SELECT ENVIRONMENTAL AND GENETIC DETERMINANTS OF ADIPONECTIN AND OBESITY IN BLACK AND WHITE WOMEN

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill
2010

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ABSTRACT

Sarah Schweitzer Cohen: Select Environmental and Genetic Determinants of Adiponectin and Obesity in Black and White Women
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Using interview data and blood samples collected at baseline from the Southern Community Cohort Study, this cross-sectional study examined serum adiponectin levels in relation to select environmental and behavioral factors. Several single nucleotide polymorphisms (SNPs) in three adiponectin-related genes (*ADIPOQ*, *ADIPOR1*, and *ADIPOR2*) were also examined in relation to adiponectin and body mass index (BMI). Multivariate linear regression models were used to evaluate the association between adiponectin and BMI separately for white and black women. Prediction models for adiponectin for black and white women were also developed using multiple linear regression. Associations between SNPs in *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* in relation to adiponectin and BMI were examined in linear regression models with adjustment for age and percentage of African ancestry to account for population stratification. Black women were found to have lower adiponectin levels compared with whites even after adjustment for body mass index (BMI). These results expand upon previous studies that were limited by small sample sizes or narrow age and body size ranges and demonstrate that racial differences in adiponectin exist across the spectrum of BMI. In the examination of predictors of adiponectin beyond BMI, the factors age, HDL-cholesterol, and hypertension were found to be strong correlates of adiponectin in both race groups. In the genetic analyses, one SNP (rs17366568) in *ADIPOQ*

was found to be significantly associated with adiponectin in white women but not in black women. This finding confirms results from two recent genome-wide association studies in European whites by demonstrating significant differences in adiponectin levels across genotypes of SNP rs17366568 and expands the current literature by examining this SNP in black women for the first time. No significant associations were observed between any of the SNPs in the three adiponectin-related genes and BMI. Observed racial differences in adiponectin and its correlates from this study will be utilized in future studies of diseases potentially affected by adiponectin such as cancer, cardiovascular disease, and type 2 diabetes. In addition, the development of lifestyle interventions as well as therapeutics that increase adiponectin levels for the purpose of disease prevention may be guided by results of this study.

To Ella, who inspires me to make the world a safer, healthier, and happier place

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ACKNOWLEDGMENTS

Raised in East Tennessee in the shadow of Oak Ridge National Laboratory, I spent my childhood believing that research was what everybody did when they grew up. I like to think that my academic journey began in those early years, listening to my dad talk to his laboratory buddies about their research at family potlucks.

I am deeply grateful for the outstanding undergraduate education I received at UNC where a flyer touting the undergraduate major in biostatistics came across my desk in Math 81 on a fall day in 1996 and set my educational trajectory in motion.

Along the way, I've had the great gift of mentorship from several outstanding individuals. Lynn Dix, David Garabrant, Lisa Signorello, and Marilie Gammon each generously shared their wisdom and supported my goals as I moved through the stages of my education and early professional development. To know each of them as not only a mentor but also as a friend is a true joy.

For the past eight years, I have been privileged to be involved with the Southern Community Cohort Study led by Bill Blot and Lisa Signorello, and I am grateful for the opportunity to work with such dedicated individuals.

My dissertation committee provided invaluable guidance and thoughtful feedback which was especially appreciated given the rapid developments in this research area.

I am grateful to my dissertation writing group including Patrick Bradshaw, Tania Desrosiers, and Sarah Nyante whose weekly cheerleading kept me going when I found

myself in the valleys inherent to the dissertation process. Emily Vavalle offered invaluable encouragement in the latter stages of writing and helped me see that balancing the completion of a dissertation and the whims of a toddler is a lifestyle that requires a willingness to laugh a lot and sleep a little.

Finally, I am grateful to Josh and Ella and to my mom, dad, and sister for the endless love and support and for the procurement of chocolate at just the right times.

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CHAPTER 1: BACKGROUND

Brief introduction to background and specific aims

The rapid increase in the prevalence of obesity in the United States in the past 30 years has many health implications. Obesity is strongly associated with incidence and mortality of several common cancers including post-menopausal breast cancer, colorectal cancer and endometrial cancer (1). In addition, obesity is a major risk factor for coronary artery disease, type 2 diabetes, hypertension, and stroke (2). Obesity patterns in the United States vary substantially by race with black women having a higher prevalence of obesity than white women (3). Additionally, black women experience higher incidence and mortality for many common cancers (4) and cardiovascular disease (5).

The proteins known as adipokines are produced largely in adipose tissue and may play a differential role in the development of obesity in black compared to white women (6, 7). Several of these proteins were discovered relatively recently and remain largely unstudied in blacks. The research presented here focused on adiponectin, an adipokine that holds great promise to elucidate links between race, obesity, and, ultimately, cancer and cardiovascular disease incidence and mortality (8, 9). A better understanding of the role of adiponectin in relation to obesity and race will provide hypotheses to be tested in the future regarding the environmental and genetic determinants of obesity as they relate to disease risk in both black and white women.

Adiponectin was first characterized in the mid-1990s. Since that time, several single nucleotide polymorphisms (SNPs) in the gene that encodes adiponectin (ADIPOQ) and the

genes encoding the adiponectin receptors (ADIPOR1 and ADIPOR2) have been identified and examined in association with measures of body size and other metabolic outcomes. However, few studies have included sufficient numbers of black subjects to assess whether the observed associations differ across racial groups. Adiponectin levels have also been shown to be negatively correlated with obesity, insulin resistance, and type 2 diabetes. Again, though, few studies have examined associations between blood levels of adiponectin and body size or metabolic disorders in black women. This work was the first to characterize environmental factors (such as physical activity, energy and nutrient intake, reproductive factors, alcohol consumption, smoking, and co-morbid conditions) and genetic polymorphisms in ADIPOQ, ADIPOR1, and ADIPOR2 in relation to adiponectin levels and obesity in a large sample of healthy black and white women.

This research included cross-sectional analyses of baseline interview data and blood samples collected at enrollment into the Southern Community Cohort Study (SCCS) from 2,000 black and white women. The SCCS is a prospective cohort study designed to understand racial disparities in cancer incidence and mortality in the southeastern United States, particularly among understudied groups such as low-income and rural residents (10). The SCCS is ideal for examining environmental and genetic risk factors for adiponectin levels and obesity as they differ by race because a considerable amount of demographic and lifestyle data as well as blood samples were collected for a large number of black and white women from similar geographic and socioeconomic backgrounds. A sample of 1,000 black and 1,000 white women, stratified by body mass index (BMI) and menopausal status, was selected from the SCCS study population to evaluate the specific aims of this study, which are outlined below.

Specific Aims

Specific Aim 1: Determine whether adiponectin levels among black women as compared to white women are associated with BMI and environmental and behavioral factors including physical activity, energy and nutrient intake, alcohol consumption, smoking, reproductive factors, and co-morbid conditions.

Specific Aim 2: Determine whether adiponectin levels among black women as compared to white women are associated with genetic polymorphisms in tag-SNPs in the ADIPOQ, ADIPOR1 and ADIPOR2 genes and, in exploratory analyses, interactions between environmental factors and genetic polymorphisms in the ADIPOQ, ADIPOR1 and ADIPOR2 genes.

Specific Aim 3: Determine whether BMI among black women as compared to white women are associated with genetic polymorphisms in tag-SNPs in the ADIPOQ, ADIPOR1 and ADIPOR2 genes; and in exploratory analyses, interactions between environmental factors and genetic polymorphisms in the ADIPOQ, ADIPOR1 and ADIPOR2 genes.

Definition and measurement of obesity

Obesity, defined at the most basic level, is simply an excess of accumulation of body fat (11). This excess results from an imbalance in energy consumption and expenditure, resulting in enlarged fat cells (also known as adipose cells) as well as an increase in the number of adipose cells (12). The major sites for adipose tissue storage are both within the abdominal cavity (known as abdominal or visceral fat) and just under the skin (known as subcutaneous fat). Visceral fat tends to accumulate with age and is more strongly associated with metabolic disorders and cardiovascular disease (13).

There is little agreement on the best way to measure obesity accurately in either clinical settings or in large-scale research studies. In epidemiologic studies, obesity is most

often measured by BMI because the component measures (height and weight) are easily obtained via self-report from study participants or from inexpensive and easy-to-use tools (11, 14). BMI is calculated as the weight in kilograms divided by the square of height in meters. Standard categories of BMI have been put forth by the World Health Organization and include underweight (BMI < 18.5 kg/m2), healthy weight (BMI 18.5-24.9 kg/m2), overweight (BMI 25.0-29.9 kg/m2), obesity class I (BMI 30.0-34.5 kg/m2), obesity class II (BMI 35.0-39.9 kg/m2) and obesity class III or extreme obesity (BMI > 40.0 kg/m2) (15). BMI has excellent validity as a measure of absolute fat mass adjusted for height (14), and the widely used, standardized cut-points established for BMI categories allow for ease of comparison across studies (15, 16). However, the calculation of BMI includes a measurement of body weight which is made up of both lean body mass and fat tissue. Thus, BMI is a less valid measure for percent body fat than other measures that account for differences in the proportion of each type of body tissue (14).

Other measures to assess body composition include densitometry (under-water weighing) as well as newer techniques such as dual energy X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), and computed tomography (CT)/magnetic resonance imaging (MRI). Densitometry requires that an individual be submerged in water. By measuring the ratio of body weight measured in air and body weight measured under water, an estimate of the proportion of fat in the total body mass can be calculated (14). DEXA uses an x-ray with low and high-energy peaks to distinguish fat mass, fat-free mass, and bone mineral mass in the whole body or by specific region (such as in the abdomen) (14). DEXA is not able to distinguish visceral fat from subcutaneous fat (17). BIA involves sending a weak electrical current through the body and measuring its impedance by muscle tissue;

because muscle is composed mainly of water and fat tissue contains virtually no water, the impedance values can be used to estimate percentage body fat (14). CT and MRI are considered to be the most accurate methods for assessing body composition including the quantification of visceral versus subcutaneous fat (17). However, these three measures all require expensive equipment, specialized technicians, and can be time-consuming to perform on a large number of individuals, and thus are not widely used in epidemiologic research.

Distribution of body fat, not just the total amount, has also been shown to be related to health risks. While fat distribution can be measured using imaging tools such as DEXA and CT/MRT, waist circumference and waist-to-hip ratio (WHR) are also used to measure differences in fat tissue distribution and has been used frequently in epidemiologic studies because the required measurements are inexpensive and quick to obtain. One limitation of these measures is that many factors can affect the measurement of waist and hip circumferences including the degree of training of the individual making the measure, the time of day, and timing of the most recent meal (14)

In addition to the limitations of the measures of obesity described above, another level of complication arises when obesity is measured in individuals of different racial or ethnic backgrounds. Reports in the literature are not entirely consistent but many studies have concluded that commonly used measures of obesity have different meanings for whites and blacks, likely due to differences in fat distribution. Several studies report that for a similar waist circumference and BMI, blacks have less visceral fat than whites (13, 18). For example, the hip circumferences of blacks have been found to be smaller than those in whites, resulting in an increased WHR for a given amount of central fat (11). Evans et al. also reported that the relationship between BMI and percent fat measured by DEXA differs

by race with black women having lower body fatness than white women at the same BMI (19). In contrast, Gallagher and colleagues found that BMI reflected the same level of fatness in black and white adults with BMI < 35 kg/m2 after age and sex adjustment (20). Differences also exist between blacks and whites with respect to fat-free body mass with blacks generally having more bone mineral density and body protein than whites (21). While tools such as under-water weighing, DEXA, and MRI are among the current gold standards for measuring body fatness, they are impractical in terms of cost and logistics for most large epidemiologic studies. Ultimately, in a large study such as the Southern Community Cohort Study, the only cost-efficient approach to measuring body size is to use measurements that are readily obtained with minimal equipment and time such as self-reported height and weight or interviewer measured height, weight, and perhaps waist and hip circumferences.

Racial differences in obesity prevalence

Based on measured height and weight data from the National Health and Nutrition Examination Surveys (NHANES), the prevalence of obesity among American adults began increasing rapidly in the late 1970s and early1980s (3). The prevalence of obesity (defined as a body mass index [BMI] > 30 kg/m2) among women in NHANES from 1988-1994 was 25.4% and increased to 33.4% in the NHANES data from 1999-2000 (22). In the 2007-2008 NHANES, the prevalence of obesity among women was 35.5%, representing a much slower increase than seen in the previous two decades (3). In addition to the rapid rate of increase in the obesity prevalence among women overall in the past thirty years, there is strong variation in the prevalence of obesity by race. NHANES data from 2007-2008 show that 33.0% of white women were obese compared to 49.6% of black women (3). In relation to the 1988-1994 estimates, this corresponds to a 10.1% prevalence increase for white women and an

11.4% increase for black women. Differential increases in the prevalence of extreme obesity (BMI > 40 kg/m2) by race were even more pronounced with the prevalence increasing from 3.4% to 6.4% among white women and from 7.9% to 14.2% among black women between the NHANES surveys covering 1998-1994 and 2007-2008 (3, 22).

Environmental and behavioral determinants of obesity

It is likely that genetic factors contribute to the ability of humans to store excess fat when food is abundant and to lose fat when food is scarce (23). However, the recent increase in obesity among American women, as well as in other populations around the globe, is unlikely to be explained solely by genetics because it has happened over such a short period of time. Thus, individual-level behavioral and environmental factors are also thought to be strong contributors to obesity including physical activity levels, energy and nutrient intake, reproductive patterns, and socioeconomic status (24-27). Population-level characteristics related to changes in occupations and infrastructure (such as changing modes of transportation) are also likely important influences on the development of obesity but are beyond the scope of this dissertation.

Physical activity

The modern environment does not require nor encourage physical activity for most adults (28). The Centers for Disease Control and the American College of Sports Medicine recommend that adults engage in at least 150 minutes per week of moderate-intensity physical activity (29) but data from the Behavioral Risk Factor Surveillance System (BRFSS) finds that more than half of US adults do not meet PA recommendations based on activity patterns in three domains (household work, transportation, and discretionary/leisure time) (29, 30). Many reasons are cited for the overall lack of physical activity among US adults including emotional barriers such as lack of time, lack of motivation, and lack of social

support as well as physical barriers such as lack of access to facilities and unsafe neighborhood conditions (31-33).

Physical activity patterns by race have been extensively examined but there is inconsistency in the literature. Many studies have found blacks are less physically active than whites (34-36), in some cases, beginning as early as adolescence (37). However, other studies have found no evidence for differences in physical activity levels across racial groups. Using data from the Health and Retirement Study, He and Baker found that leisure-time physical activity did not differ between blacks and whites after adjustment for education and health status (38). Similarly, Marshall and colleagues found that within strata of social class (including education, income, employment status, and marital status), there were few differences in the prevalence of physical inactivity between white and black women (39).

Energy and nutrient intake

Energy intake that exceeds the energy needs of the body has been shown in controlled studies to cause weight gain in the form of stored fat (40). However, the role of particular dietary factors as determinants of obesity is much less clear. Several methodological problems have been identified in studies of diet and obesity including short time periods of measurement, correlations between dietary factors and other determinants of obesity such as physical activity, and the validity and reliability of the tools used to measure dietary intakes (40). Despite these limitations, ecologic, observational, and intervention studies have identified links between obesity and the consumption of fats, high-fructose corn syrup, fast food, and snack foods (41-44). Recently, Drewnowski set forth a single explanation for these findings, namely that the consumption of low-cost foods which contain refined grains, added sugars, and added fats, explain the many links observed between weight gain and individual foods on a population level (45). This hypothesis is consistent with the increased risk of overweight

and obesity among black women who are disproportionately of lower SES than white women.

Reproductive factors

There may also be important racial differences in reproductive factors that affect the prevalence of obesity. While the role of parity in the development of obesity remains somewhat uncertain (46, 47), several studies have indicated that increasing parity is associated with an increase, albeit modest, in the risk of obesity (48-52). In the few studies with sizable numbers of black women, it has been observed that black women appear to be more susceptible to weight gain following pregnancy than white women (53, 54). For example, black women were found to retain more weight post-partum than white women at similar levels of gestational weight gain (53). A recent analysis that stratified women by metropolitan status found that the effect of increased parity was significant only in black women living in metropolitan areas but not black women living in non-metropolitan areas (55). A cross-sectional study using data from the Southern Community Cohort Study found a modest increase in the odds of obesity among both white and black women having five or more births compared to nulliparous women (56). In addition, black women have more children on average than white women (57) and some studies have indicated that high levels of parity are most strongly associated with obesity (49, 50, 58). Further, differences in the prevalence and length of breast-feeding exist with black women being less likely to breastfeed compared to white women (59, 60). Some studies have reported that breast-feeding is associated with a small decrease in weight retention post-partum (61, 62) which in combination with a lower prevalence of breastfeeding among blacks could contribute to the disparity in the prevalence of obesity.

Smoking

A strong negative correlation has been consistently observed between cigarette smoking rates and obesity (63). This association is explained by the appetite suppressing effect of nicotine, one of many components in tobacco. By suppressing appetite, smokers are believed to decrease food intake and thus have lower overall body size compared to non-smokers (63).

Alcohol consumption

Mechanistically, alcohol consumption is thought to lead to an increase in food intake because of its relatively high contribution of kilocalories (7 kcal/g), its relaxing effect on the nervous system, and its minimal effect on satiety (64). While most epidemiologic studies have shown that energy from alcohol is added to energy from food intake without any compensation for the extra calories provided by alcohol, associations between alcohol intake and obesity have been observed less consistently (65). Among British men, increasing alcohol intake was associated with general obesity (as measured by BMI) and even more strongly with central obesity (as measured by waist-to-hip ratio and waist circumference) (66). These results, in light of conflicting reports in the literature regarding associations between obesity and alcohol indicate a need for future research based on fat distribution and alcohol consumption.

Socioeconomic status

Underlying many of the observed associations between environmental and behavioral characteristics and obesity is the issue of socioeconomic status (SES). In a descriptive review of the literature regarding obesity and SES, McLaren reported that in resource-rich countries, such as the US, lower SES was associated with larger body size among women in nearly two-thirds of the reviewed studies (67). Racial differences in BMI have been found to be only partially explained by measures of SES such as education and income (68-71). Wang and Beydoun (69) hypothesize that a major reason for this finding is that factors such

as body image, lifestyle, social structure, and physical environment are responsible for much of the racial difference in body size and that these constructs are not adequately accounted for by adjustment for standard SES measures such as education and income. Clearly, careful attention to measurement and adjustment for SES is imperative in untangling associations between race, obesity, and environmental and behavioral characteristics.

Summary

Links between Individual-level behavioral and environmental determinants of obesity are depicted in Figure 1.1. As described above, many factors affect obesity in adults and these factors are often associated with one another.

Genetic determinants of obesity

Given that the prevalence of obesity has risen quickly in only a few decades, it is likely that environmental factors are largely responsible. However, well before the occurrence of the recent rapid increase in the prevalence of obesity, a small but stable proportion of the population was obese. In the NHANES from 1960-1962, the prevalence of obesity among women age 20-74 was 15.8%. A decade later, in NHANES 1, the prevalence was only slightly higher at 16.6% (22). The presence of a small but consistent group of individuals with increased body size that existed before the pro-obesigenic environmental changes in recent decades indicates that genetics plays an important role in the development of obesity in at least some individuals.

Further strengthening the hypothesis that obesity has strong genetic determinants is that obesity has been found to be highly heritable. A recent review of the literature regarding the genetic epidemiology of obesity reports heritability estimates from twin, adoption, and family studies ranging from 16 to 85% for BMI (72). Thus, the genetic underpinnings of obesity are an important component of a complete understanding of obesity. Genetic variants

may also play an important role in determining which individuals are susceptible to obesity in an environment of abundant, energy-dense food and low physical activity levels (23, 72, 73).

Common obesity (as opposed to obesity related to a rare Mendelian disorder) is an example of a complex disease that may follow either the 'common disease/common variant' (CD/CV) paradigm or the 'common disease/rare variant' (CD/RV) paradigm, and the scientific community remains divided as to which scenario best describes the genetic underpinnings of obesity (74). The CD/CV paradigm says that common, complex diseases are likely due to disease loci which have one or only a few variants that occur with a high frequency in the population (75-78). The CD/RV paradigm is similar to the CD/CV paradigm in that both are attempts to explain the genetic basis of complex diseases and both recognize that multiple disease loci are likely to be involved. However, the CD/RV paradigm says that instead of disease risk being due to disease loci with a few common variants occurring at high frequency in the population, disease risk is due instead to many loci each with multiple variants with low population frequency (74, 77). The CD/CV paradigm suggests that common mutations are generally found in multiple human populations while the CD/RV paradigm suggests that mutations may be population-specific (76). Depending on the true underlying genetic structure of obesity, different types of studies (linkage versus association) will have varying degrees of success. For example, association studies are likely to be successful only if the CD/CV paradigm is correct because effect estimates for disease risk are modest in magnitude and require ample sample size for the statistical signal to be detected (76, 77).

Loci linked to BMI have been found on all chromosomes except Y via linkage studies (including genome-wide linkage studies); however, very few regions have been replicated (72, 79). Linkage analysis has proven very useful for identifying genes for monogenetic diseases but limited power and the challenges of obtaining family-based data have contributed to less success for linkage studies of common complex diseases (72); thus few validated genes for obesity-related phenotypes have been identified via linkage studies. In contrast, the vast majority of studies showing associations between obesity and specific genes have been association studies (72). In a 2007 review of the genetic epidemiology of obesity, Yang et al. report that 426 findings of positive associations in 127 candidate genes have been identified to date, and that 22 of these genes have been supported by at least five positive studies (72). The advent of genome-wide association studies (in which several hundred thousand SNPs are genotyped for each study subject and examined for association with the outcome of interest) has increased the identification of potentially important genes in relation to obesity that were not previously identified by the candidate gene approach. For example, in 2007, FTO, a gene not previously identified as being associated with obesity, was identified via a genome-wide association study (80) and replicated by other groups (81, 82).

Lack of replication in both linkage and association studies is a serious concern and may be due to issues such as high rates of false positives, lack of amply powered study samples, and insufficient attention to the potential for population stratification (72). Relatively small sample sizes have precluded previous studies from being able to examine gene-gene and gene-environment interactions (such as with physical activity and dietary

patterns), limiting our ability to understand fully the role that genetics plays in the development of obesity (72).

Associations between obesity and chronic disease

Cancer risk

This project sought to further our understanding of associations between environmental and genetic factors and obesity. Long range, an important goal of the SCCS is to gain a better understanding of cancer risk as it relates to obesity in different racial groups because obesity is one of the few modifiable risk factors for many cancer sites. This work is particularly important because of the higher prevalence of overweight and obesity among black women and observed disparities in cancer mortality for several common sites. For example, the mortality rate for breast cancer in the US in 2005 was 23.3 per 100,000 women among whites and 32.8 per 100,000 women among blacks (83). Similarly for colorectal cancer, the mortality rate among whites in 2005 was 14.1 per 100,000 compared to 21.2 per 100,000 among blacks (83). This project was unique because with obesity as a focus, the results of this study will assist in the generation of research hypotheses to be tested in the future in the SCCS and other studies in relation to multiple cancer sites. Thus a brief description of obesity and a select group of cancers based on either population burden or strength of evidence are provided here.

Obesity is associated with incidence and mortality for several of the most common cancer sites (1, 84-86). In the Cancer Prevention Study II (CPS II), including 495,477 women followed for 16 years, the relative risk for all cancer mortality was 1.62 (1.40-1.87) comparing women with a BMI of > 40 kg/m2 to women with a BMI between 18.5 and 24.9 kg/m2 (86). Among the women in the CPS II, obesity was found to be associated with cancer of the esophagus, colon and rectum, liver, gallbladder, pancreas, kidney, breast,

uterus, cervix, and ovaries with RRs ranging from 1.46 (0.94-2.24) for colorectal cancer to 6.25 (3.75-10.42) for the uterus (86). The 2002 report on Cancer Prevention, Weight Control, and Physical Activity from the International Agency for Research on Cancer (IARC) concludes that, based on a comprehensive evaluation of the literature, there is sufficient evidence for a cancer-preventive effect of avoidance of weight gain for colon, post-menopausal breast cancer, endometrial cancer, kidney, and esophageal cancers (87).

Obesity is an established risk factor for breast cancer (88) and the association has been found to differ by menopausal status (89). Among premenopausal women, obesity has generally been found to be inversely associated with breast cancer risk (89, 90). In a meta-analysis, Ursin and colleagues found reductions in the relative risk (RR) for breast cancer among four cohort studies (RR for 8 kg/m2 reduction in BMI=0.70, 95% CI=0.54-0.91) and 19 case-control studies (RR for 8 kg/m2 reduction in BMI=0.88, 95% CI=0.76-1.02) although the individual study estimates were quite heterogeneous (91). More recently, in the Pooling Project of Diet and Cancer, a pooled analysis of seven large prospective studies, the RR for breast cancer among women with a BMI > 33 kg/m2 was 0.58 (95% CI=0.34-1.00) compared to women with a BMI < 21 kg/m2. A similar reduction in the RR was seen for women with a BMI between 31 and 33 but not for women with a BMI below 31 (92). Additional studies have found similar results although variation is evident when different measures of obesity, such as waist-to-hip ratio versus BMI, are considered (89). Notably, these studies have almost entirely been conducted in white women (93).

In contrast, among postmenopausal women, increased body size is positively associated with breast cancer risk (89, 90). In the same pooling project analysis described above, the RR for breast cancer among postmenopausal women with a BMI greater than 28

kg/m2 was 1.26 (95% CI=1.09-1.46) compared to women with a BMI < 21 kg/m2. A stronger positive association with obesity was seen among women who had never used hormone replacement therapy (HRT) (92). In the Women's Health Initiative cohort, evidence of effect measure modification by HRT was also seen with obesity found to be a risk factor for breast cancer among non-users of HRT but not among women who had ever used HRT (94). Positive associations between adult weight gain and breast cancer risk as well as central adiposity and breast cancer risk have been consistently reported as well (89, 90).

Consistent evidence also exists to link colorectal cancer and obesity (1). In the 2002 IARC report, relative risks for colorectal cancer in obese women were reported to be approximately 1.2-1.5 (87). The observed relative risks are generally lower in women than in men (1, 95) but the coupling of colorectal cancer as the third most common incident cancer among women (4) and the high prevalence of obesity in the United States makes even modest relative risks such as these an important public health concern. Some authors have suggested that the weak associations seen for women are due to effect measure modification by menopausal status or estrogenic effects (96, 97). In a cohort of nearly 90,000 women in Canada, the RR for colorectal cancer in the entire cohort was 1.08 (95% CI 0.82-1.41) but differed significantly for premenopausal women (RR=1.88, 95% CI=1.24-2.86) and post-menopausal women (RR=0.73, 95% CI=0.48-1.10) (96). Similar results were seen in a population-based case-control study of colon cancer in California, Utah, and Minnesota with an RR of 2.19 (95% CI=0.94-5.07) for premenopausal women (comparing women with a BMI >30 to women with a BMI <23) and an RR of 1.29 (95% CI=0.93-1.73) for postmenopausal women. These authors also stratified women based on estrogen status (with

positive status describing women who were either premenopausal or were postmenopausal and taking HRT, and negative status including postmenopausal women not taking HRT). Estrogen-positive women had an RR of 2.50 (95% CI=1.51-4.13) for colorectal cancer while estrogen-negative women had an adjusted RR of 0.96 (95% CI=0.65-1.41) (97). The possibility of effect measure modification by menopausal status or estrogen level has been inconsistent, though. In the Women's Health Study which examined nearly 40,000 women, BMI was found to be positively associated with increased colorectal risk overall. However, in analyses of postmenopausal women, the relationship was not modified by estrogen exposure (98).

In a 2005 review of all English-language literature published between 1969 and 2004, Modessitt and colleagues found that the most consistent finding linking obesity and cancer is for endometrial cancer (99). These authors report a three- to tenfold increase in endometrial cancer risk associated with obesity. In a larger review of obesity and cancer, Calle and Thun reported that evidence from both case-control and cohort studies showed an increase in risk of endometrial cancer for overweight or obesity to be in the range of two- to fourfold (1). Two major types of endometrial cancer are distinguishable with the more common (80%) endometrioid carcinomas being more strongly associated with obesity than the rarer serous papillary, clear cell, or squamous carcinomas (100). However, many studies have not been able to examine associations with obesity by subtype (99, 100).

Cardiovascular disease risk

Obesity is a major risk factor for both cardiovascular disease (CVD) morbidity and mortality. Despite some controversy regarding the exact shape of the overall BMI-mortality curve, the preponderance of evidence points to an elevated risk of death from CVD among obese individuals. Using NHANES I,II, and III data, Flegal et al. reported relative risks that were

consistently above 1.0 for all cardiovascular disease mortality among obese (BMI > 30 kg/m2) individuals (101). These authors attributed between 9 and 13% of total CVD mortality to obesity although they noted that the association between CVD mortality and obesity may have been decreasing over time (101). In the Framingham Heart Study, similarly elevated relative risks for cardiovascular death were found among obese women (RR=1.56, 95% CI=1.00-2.43) although not among obese men (RR=0.98, 95% CI=0.59-1.63) (102). McGee and colleagues in the Diverse Populations Collaboration examined associations between obesity and mortality from coronary heart disease (CHD) and all cardiovascular disease (CVD) using meta-analytic techniques and person-level data from 26 observational studies (103). Relative risks comparing obese to healthy weight women were 1.62 (95% CI 1.46-1.81) for CHD mortality and 1.53 (95% CI 1.38-1.69) for CVD mortality. Similar elevations were observed among males (103).

Obesity is also associated with incident CVD including heart disease, the leading cause of death in the United States, and stroke, the third-leading cause of death (57). Among women in the NHANES III sample, obesity classes 1 (BMI 30-34.9 kg/m2), 2 (BMI 35-39.9 kg/m2) and 3 (BMI 40+ kg/m2) were associated with increased prevalence ratios for coronary heart disease ranging from 1.6 (for obesity class 1) to nearly 3.0 (for obesity class 3) (104). Using incident CVD, obese women in the Framingham Heart Study cohort were found to have elevated risk of total cardiovascular disease (RR=1.39, 95% CI=1.14-1.68) as well as increased risk of angina pectoris (RR=1.63, 95% CI=1.18-2.25) and myocardial infarction (RR=1.46, 95% CI=0.94-2.28); however, no association was seen with cerebrovascular disease (102). Congestive heart failure and atrial fibrillation have also been found to be associated with obesity (105).

The association between obesity and cardiovascular disease is mediated in part via CVD risk factors that are highly associated with obesity including hypertension, insulin resistance, and dyslipidemia (23). Many studies have found associations between obesity and type 2 diabetes. For example, among women in the Nurses Health Study, the relative risk of type 2 diabetes over 14 years of follow-up among women with a baseline BMI greater than 31 kg/m2 was in excess of 40 times higher than for women with a baseline BMI less than 22 kg/m2 (106). In the Framingham cohort, obesity was associated with both hypertension (RR=2.63, 95% CI 2.20-3.15) and diabetes (RR=1.36, 95% CI=1.03-1.78) among women (102). Additional studies have found strong positive associations between increasing blood pressure and BMI (105). For example, in NHANES III, strong associations between high blood pressure and obesity were found among women less than 55 years of age with prevalence ratios ranging from 1.65 (for overweight) to 5.45 (for obesity class III). Among women over 55, hypertension was still associated with increasing BMI category although the prevalence ratios were smaller in magnitude (1.41 for obesity class III, for example) (104). High cholesterol level was also found to be associated with increasing category of BMI among women less than age 55 in the NHANES III sample with prevalence ratios around 1.7 for obesity classes 1, 2, and 3 (104).

Biologic mechanism linking obesity to cancer and cardiovascular disease

While not exhaustive, several of the important obesity-mediated biologic pathways to cancer and cardiovascular disease (CVD) are depicted in Figure 1.2. Obesity is linked to cancer through several pathways, including the insulin resistance pathway (1, 87). With an increase in adipose tissue, other body tissues become insensitive to insulin which triggers the production of larger amounts of insulin, ultimately resulting in a state of hyperinsulinemia. Chronic hyperinsulinemia causes a decrease in insulin-like growth factor binding proteins

(IGFBP-1 and IGFBP-2) which in turn results in higher levels of free IGF-1 in the blood. This is important because IGF both stimulates cell proliferation and inhibits apoptosis, two processes that are involved in carcinogenesis. Sex hormone-binding globulin (SHBG) synthesis is reduced by elevated levels of free IGF-1 as well as by chronic hyperinsulinemia directly. SHBG is a binding site for estrogen and thus lowered levels of SHBG increase the amount of unbound estrogen in the blood. Estrogen is a known promoter of carcinogenesis due to its pro-cell proliferation and anti-apoptotic qualities.

In addition to the insulin-IGF-SHBG pathway, obesity directly affects estrogen levels and thus increases the risk of hormone-sensitive cancer sites such as breast and endometrium (107). In particular, in post-menopausal women, the principal source of estrogen is the conversion of androstenedione to estrone in the adipose tissue. The insulin resistance pathway also links obesity to CVD due to the strong association between chronic hyperinsulinemia and type 2 diabetes, which is both a CVD endpoint as well as a risk factor for other CVD events such as coronary heart disease (108).

In addition to the insulin resistance pathway, obesity is linked to carcinogenesis as well as CVD via the inflammation pathway. As will be described further in the next section of this work, adiponectin is inversely associated with obesity. Decreased adiponectin levels result in the activation and proliferation of immune cells and inflammatory cytokines such as TNF-α (109). These cells contribute to a state of chronic inflammation. Carcinogenesis is thought to result from the chronic inflammatory state as a result of the effects of sustained tissue damage and damage-induced cell proliferation (110). Decreased adiponectin levels also may act directly on cancer cells by decreasing cell arrest and apoptosis while increasing cell proliferation (111).

Obesity is also hypothesized to be linked to cancer via inflammation with cyclooxygenase-2 (COX-2) being one of the likely mediators. COX-2 is up-regulated at sites of inflammation, likely by the inflammatory cytokines, growth factors, and tumor promoters (112). The increased levels of COX-2 inhibit apoptosis as well as promote angiogenesis leading to carcinogenesis (112, 113).

Decreased levels of adiponectin are also directly related to the formation of atherosclerotic plaques by down-regulating vascular adhesion molecules and inhibiting the transformation of macrophages to foam cells (6). The formation of atherosclerosis is a major risk factor for coronary artery disease and other CVD events. Other cytokines such as interleukin-6 (IL-6) and CRP are also believed to be intermediates in the pathway between obesity, CVD risk factors (such as hypertension) and CVD outcomes (105).

While the pathways discussed above are hypothesized to be important links between obesity and cancer and CVD, their relative importance is yet to be determined in relation to one another as well as to other pathways not yet explored. Importantly, it appears that the cytokines, including adiponectin, may play an important role in several pathways linking obesity and chronic disease.

Biology of adiponectin

Adiponectin protein

Adipose tissue was traditionally considered to be an energy storage organ, but beginning in the early 1990s, it was discovered that adipose tissue also functions as an endocrine organ, secreting proteins that affect multiple metabolic pathways (114). These proteins are alternatively called cytokines, adipokines, and adipocytokines. One such protein, adiponectin, was independently characterized by four different laboratories in the mid-1990s

(115-118) but did not receive the type of scientific attention that was given to leptin until the late 1990s to early 2000s (6).

Adiponectin is produced exclusively in adipose tissue and is found in a high concentration in the blood, accounting for approximately 0.01-0.05% of total serum protein (6, 114). Four domains including a total of 247 amino acids make up adiponectin including an amino-terminal signal sequence, a variable region, a collagenous domain, and a carboxy-terminal globular domain (called "globular adiponectin") (119). In circulation, adiponectin is found to be almost entirely full-length although a small amount of globular adiponectin is also present (120). Adiponectin is synthesized as single units which subsequently bond with other units to form higher order multimers (or isoforms) that are secreted into the bloodstream (121). Adiponectin is present in serum and plasma in several of these higher-order forms including as a trimer (formed by three single units which bond in a ball and stick-like structure), a hexamer (which consists of two trimers bonded in a head-to-head manner via disulphide bond) and as higher-order structures called high molecular weight (HMW) adiponectin (9, 121). The HMW forms appear to consist mainly of 18 subunits (six trimers) which form either a flat fan-shape or a bouquet-like structure (121). The specific isoform is thought to influence the biological activity of adiponectin through differential activation of downstream pathways although more research is needed to understand the specific actions of the different isoforms (122). Recent data indicates that the HMW isoform binds most avidly to its receptors (123) and may be more strongly associated with insulin resistance and the metabolic syndrome (123-125).

Adiponectin levels appear to be relatively stable within individuals who do not undergo drastic changes in body weight. Circadian variation has been shown to be low

overall with adiponectin levels varying less than 20% throughout the day, and slightly more variation seen in females than in males (126). Most evidence seems to indicate that adiponectin levels remain unchanged in relation to meal ingestion (126). Among 48 Chinese males, adiponectin levels measured during four seasons over one year apart were highly correlated (intra-class correlation coefficient [ICC]=0.81) (127). In another study of 300 white men, in blood samples collected one year apart, adiponectin levels had a similarly high ICC of 0.85 (128).

In a 2005 review of recent progress made in adiponectin research, Kadowaki and Yamauchi put forth an "adiponectin hypothesis" in which they postulate that reduced adiponectin levels result from interactions between SNPs in the adiponectin-encoding gene, environmental factors such as diet, and down-regulation of adiponectin receptors linked to obesity. The resulting reduction in adiponectin levels then plays a causal role in the development of insulin resistance, type 2 diabetes, metabolic syndrome, and atherosclerosis (120). Because increased levels of adiponectin are inversely associated with obesity phenotypes as well as several obesity-related disease outcomes, adiponectin may be a useful therapeutic tool in the treatment of insulin resistance, type 2 diabetes, obesity, cardiovascular disease, and cancer; however, to date, most studies that have demonstrated a reduction in these morbidities after administration of adiponectin have been in rodents (6, 119, 120).

Adiponectin receptors

Two adiponectin receptors have been identified to date, AdipoR1 and AdipoR2 (6, 120). These receptors are found in the cell membrane and are located throughout the body in liver, muscle, and adipose tissue although AdipoR1 is found predominantly in muscle cells while AdipoR2 is primarily found in the liver (129). AdipoR1 has the highest affinity for globular adiponectin (which accounts for only a small proportion of circulating adiponectin) while

AdipoR2 binds both globular and full-length adiponectin (130). The binding of adiponectin to these receptors mediates the activation of AMP kinase which leads to expression of peroxisome proliferators-activated receptor-alpha (PPAR-α) (120). This activity is believed to increase gene expression of enzymes related to fatty acid oxidation and glucose uptake (6, 120). This process is thought to be one of the main mechanisms linking adiponectin and insulin sensitivity. Additionally, increased obesity is thought to either directly decrease expression levels of adiponectin receptors or reduce the post-receptor signaling which may also contribute to insulin resistance (6). T-cadherin, a calcium-dependent transmembrane adhesion protein, has also been found to be a third receptor for full-length adiponectin in vascular endothelial cells and smooth muscle (6, 129).

Genes encoding adiponectin and adiponectin receptors

The human adiponectin gene, known as ADIPOQ, has been identified and is located on chromosome 3q27 (8). ADIPOQ is approximately 17,000 base pairs (bp) in length and consists of three exons and two introns (131). The genes coding for the adiponectin receptors (ADIPOR1 and ADIPOR2) have also been identified. The adiponectin receptor 1 gene (ADIPOR1) is located on chromosome 1q32 and is approximately 17,000 bp in length. The gene encoding adiponectin receptor 2 (ADIPOR2) is located on chromosome 12q13 and is approximately 97,000 bp (132).

Race and gender differences in adiponectin levels

Gender differences in adiponectin levels have been observed in many studies. Most studies have found that women have higher adiponectin levels than men even after adjustment for body size and fat distribution (133-138). One small study of prepubescent children found no evidence for gender differences in adiponectin levels (134) but little evidence indicates this pattern is true in adults. Gender differences are hypothesized to exist due to multiple factors.

Increased levels of HMW adiponectin among women are thought to contribute to gender differences in measures of total adiponectin (139). In addition, inhibition of adiponectin production from circulating androgens is also thought to keep adiponectin levels lower in males (126).

Racial differences in adiponectin levels have been examined in a relatively small number of studies most of which had low sample sizes; however, reports have been generally consistent that adiponectin levels are lower in blacks compared to whites and the differences emerge relatively early in life (134, 140). Two small studies of children and early adolescents both reported that adiponectin levels were lower in blacks compared to whites in both genders (134) and among boys after matching on BMI percentile (140). Two additional studies of middle-age adults reported lower adiponectin levels in American blacks (N=212) (141) and in African blacks (N=27) (136) compared to whites. Two larger cohorts of young (age 23-45) and middle-aged (age 48-58) adults also reported lower adiponectin levels in black participants compared to white participants (137, 142). In a large study of older adults (age 70-79), adiponectin levels were found to be lower in blacks (N=1044) compared to whites (N=1429) (143). Hulver and colleagues (144) reported mean adiponectin levels in strata of race and obesity status. They found that mean adiponectin levels were similar in obese white women, obese black women, and non-obese black women but higher in non-obese white women (144). In a study of white and black South Africans, adiponectin levels were lower in normal weight blacks compared to whites but no differences were seen for overweight and obese women (145). Both of these studies to examine adiponectin levels over categories of body size were limited by their small overall sample size (N=85 and N=217, respectively). Wassel Fyr and colleagues measured the percentage of European

ancestry using 35 ancestry informative markers in a sample of 1241 older adults (age 70-79) who self-reported as black. In models adjusted for adiposity, fasting glucose levels, insulin levels, blood pressure, and lipids, increasing adiponectin levels were found to increase as the percentage of European ancestry increased (146). This pattern is consistent with the previous reports described above that found lower adiponectin levels among blacks compared to whites using self-report.

Environmental and behavioral factors correlates of adiponectin levels

Dietary factors and weight loss. The effect of specific dietary factors on adiponectin levels has not been carefully examined to date; however, there are several studies of weight loss in relation to adiponectin levels. Among individuals who have lost significant amounts of body mass either through long-term restricted diets or surgical intervention, adiponectin levels have been shown to increase (147). However, in studies of moderate weight loss through traditional diet or exercise interventions, changes in adiponectin levels have been smaller or non-existent (147). Adiponectin levels also do not appear to respond acutely to meal size or composition (147). The earliest study to examine total calorie as well as macronutrient intake yielded no associations with adiponectin (148). Fiber has reported to be positively associated with adiponectin among diabetics (149, 150), a finding which is consistent with known inverse associations between both fiber intake and glucose levels and adiponectin levels and glucose levels due to reduced glucose output in the liver related to adiponectin action (147).

Alcohol. Alcohol is known to be associated, through moderate consumption, with improved insulin sensitivity and cardiovascular outcomes (151). Adiponectin is hypothesized to be an intermediary in the chain of effects linking alcohol to these outcomes via several pathways including the reduction of TNF-α, reduction in liver glucose production, and increased

muscle fat oxidation (152). Several small studies have demonstrated increased adiponectin levels in relation to increased alcohol consumption (152-154) and it has also been suggested that different types of alcohol (i.e. beer, wine, or liquor) have differing effects on adiponectin levels (153).

Physical activity. Physical activity has been examined in relation to adiponectin levels in several cross-sectional studies as well as intervention trials but results have been conflicting (155, 156). A recent review of 33 of these studies found generally that exercise increases adiponectin levels, perhaps in a dose-response relationship with moderate to high intensity exercise showing stronger effects than low intensity activity (155).

Cigarette smoking. Cigarette smoking has been associated with reduced adiponectin levels in previous studies but the mechanism is unclear (74, 157). Direct action of nicotine on adipocytes is one hypothesis to explain this inverse relationship as are less direct results of cigarette smoking including inflammation, tissue hypoxia, and sympathetic nervous system action related to nicotine receptors (135, 157).

Associations between adiponectin levels and chronic disease

Adiponectin levels and obesity

Serum adiponectin levels are negatively correlated with body mass index and waist-to-hip ratio (WHR) (6, 114) which reflect overall adiposity and fat distribution, respectively. This is somewhat paradoxical given that most cytokines (such as leptin) increase directly in relation to body fat. It has been hypothesized that feedback loops exist between obesity, adiponectin expression, and regulation of the adiponectin receptors, resulting in the observed inverse association between obesity phenotypes and adiponectin levels in the blood (119, 120).

Most evidence to date regarding the obesity-adiponectin relationship has been observed in white or Japanese populations (133, 135, 138, 158). Despite the known differences in the prevalence of obesity and risk for obesity-related disease, a relatively low number of studies have examined the relationship between adiponectin and obesity in blacks and many have had very small sample sizes. In a study of adolescents including 40 white and 46 black participants, Degawa-Yamauchi et al. observed that adiponectin was negatively correlated with both BMI and percentiles of BMI (140). In another small study, Hulver et al. found that adiponectin was negatively correlated with BMI only among whites (N=48) but not blacks (N=37) (144) while, in contrast, Araneta et al. found that adiponectin was negatively associated with increasing tertiles of BMI, waist girth, and WHR in both blacks (N=212) and whites (N=143) (141). Comparing black and white South Africans, adiponectin levels were found to be negatively correlated with BMI in each race group in univariate analysis although not in the final multivariate model (145). In a genetically homogeneous sample of 431 individuals from 7 families living on the Caribbean island of Tobago, adiponectin was also found to be negatively correlated with BMI (159).

Only a few large studies to date have examined associations with body size in populations with a sizeable number of black participants. Among 522 black participants in the Insulin Resistance Atherosclerosis (IRAS) Family Study, visceral adipose tissue measured by CT was strongly negatively correlated with adiponectin levels (160). In the Atherosclerosis Risk in Communities Study (ARIC), mean adiponectin levels were found to decrease over categories of BMI (<25, 25-<30, and 30+ kg/m2) in 630 black and 523 white participants age 48-58 (132). Further, the adjusted mean adiponectin values were lower for black women than for white women in each BMI category (142). Waist circumference was

found to be negatively associated with adiponectin levels in the CARDIA study which included 1615 white and 1360 black young adults (age 23-45) (137).

Adiponectin levels and cancer

The process of understanding the complex relationships linking obesity, the adipokines, and cancer is an evolving and very active field of research (8). Here I review the studies conducted to date regarding adiponectin levels in major cancer sites that affect women. In the cancer literature, only in the most recent years have research teams begun to examine associations between adiponectin and breast cancer both in vitro and in vivo. In vitro studies have examined the effects of adiponectin on epithelial breast tissue and on breast cancer cell lines. MCF-7 breast cancer cells were found to express functional adiponectin receptors in several in vitro studies (161-164). Conflicting evidence exists as to whether breast cancer cell proliferation is inhibited by adiponectin with some groups finding evidence for this activity in vitro (161, 164, 165) while others have been unable to replicate this finding (162, 163). Additionally, several tumor cell lines have been shown to express the adiponectin receptors AdipoR1 and AdipoR2 indicating that adiponectin could act directly on cancer cells through signaling of its receptors (166).

To date, at least seven studies in human populations have examined associations between adiponectin and breast cancer risk. Five relatively small case-control studies conducted in women residing in Japan, Greece, and Taiwan found a reduced risk of breast cancer at the highest levels of adiponectin compared to the lowest levels (167-171). In two studies (167, 170), the results were consistent between pre- and post-menopausal women while two others found an association only among post-menopausal women (169, 171). The Japanese study also found that lower adiponectin levels were associated with larger tumors and higher grade tumors but these results have yet to be replicated (170). A fifth case-control

study, conducted in Korea, found no association between tertiles of adiponectin and breast cancer risk (OR=0.92, 95% CI=0.46-1.81) (172). The Nurses Health Study used pre-diagnosis blood samples for a prospective case-control study including 1,477 cases and 2,196 controls (173). These authors found that breast cancer risk was reduced when comparing the highest quartile of adiponectin to the lowest among post-menopausal women (OR=0.73, 95% CI=0.55-0.98) but not among pre-menopausal women (173). With the exception of the Nurses Health Study, the remaining case-control studies to have examined adiponectin levels in relation to breast cancer used blood samples collected post-diagnosis. Without a clear understanding of the determinants of adiponectin levels, the measurement of adiponectin in blood samples in women after a cancer diagnosis has been made has serious implications for potential bias and is an important limitation. Notably, none of these studies included any appreciable numbers of women of African descent.

Scant literature is available examining potential associations between adiponectin and colorectal cancer. In one laboratory study, adiponectin was found to increase proliferation and increase inflammatory actions in human colon cells (174), and in another, adiponectin was found to inhibit colorectal cancer cell growth (175). Observational study results are few and thus far inconsistent. Among men in the Health Professionals Follow-up study, colorectal cancer risk was reduced when comparing the highest to the lowest quartile of serum adiponectin (RR=0.50, 95% CI=0.26-0.97) (176) but in a case-control study (also among males) in Norway and Sweden the RR for highest to lowest quartile of serum adiponectin was 0.9 (95% CI=0.6-1.4) (177). Two additional small case-control studies found lower adiponectin levels in colorectal cancer patients compared to controls (178, 179).

Notably, there have been no adequately powered studies to examine adiponectin levels in relation to colorectal cancer among women or among blacks.

In one small study in Japan, adiponectin receptors were found to be expressed in healthy endometrial tissue (180). Adiponectin suppressed cell proliferation in two endometrial carcinoma cell lines via induction of cell cycle arrest and apoptosis in another (181), but, generally, few in vitro studies have been conducted examining the effects of adiponectin on either healthy or cancerous endometrial tissue. Several case-control studies have examined the association between adiponectin in serum or plasma and endometrial cancer in women. While each study categorized adiponectin differently (including by tertiles, quartiles, and standard deviations), the collective results point to an inverse association between increasing adiponectin level and risk of endometrial cancer (182-186). Only one study included any black women but the proportion was small (15% of 117 cases) and no race-specific analyses were conducted (185).

Adiponectin levels and diabetes

Adiponectin has been examined in relation to several measures related to glucose sensitivity and insulin action including the homeostasis model assessment of insulin resistance (HOMA), insulin sensitivity, and fasting glucose levels. Both black and white participants were included in at least six studies, several relatively small, examining adiponectin levels in relation to HOMA. Four reports found that adiponectin was negatively associated with HOMA in all study participants (137, 140, 141, 145) while two others found evidence for a negative association with HOMA only in white participants (136, 144). Another study comprised only of black participants found HOMA to be negatively associated with adiponectin levels in univariate analysis but not in the final multivariate model (187). Several authors have found evidence for a positive association between insulin sensitivity

and adiponectin levels including among a small group of black and white children (134), in a small convenience sample of unknown racial background (135), and in a large family-based study of Hispanics and blacks (160). The association between adiponectin levels and fasting glucose has been found to be negative in at least one large study (160) but not in two other smaller studies (140, 144).

Incident type 2 diabetes in relation to adiponectin levels was examined in a 2009 meta-analysis that included thirteen prospective studies (188). Nearly 15,000 participants were included in the meta-analysis and the RR for type 2 diabetes overall was found to 0.72 (95% CI=0.67-0.78) for each 1-log ug/ml increase in adiponectin level. Of the studies included in this meta-analysis, two included black participants (142, 189). Using ARIC data, Duncan et al. reported an odds ratio of 0.58 (0.34-0.99) for incident type 2 diabetes comparing the highest quartile of adiponectin to the lowest quartile in a combined group of blacks (N=523) and whites (N=630). This association was similar by race although there was some suggestion that the magnitude of effect may be larger in blacks than in whites (142). In contrast, among a combined group of blacks (N=905) and whites (N=1451) age 70-79, adiponectin levels were not found to be associated with incident type 2 diabetes (RR=1.04 (95% CI=0.69-1.56)) but no race-stratified estimates were provided (189).

Adiponectin levels and cardiovascular disease

Adiponectin levels have been examined in relation to cardiovascular disease (CVD) as well as in relation to several risk factors for CVD including HDL cholesterol levels and hypertension. Adiponectin was positively correlated with HDL cholesterol in studies of whites (135, 158) and blacks (160). The HMW form of adiponectin may be more strongly associated with HDL-cholesterol (190). Previous work including both cross-sectional and prospective studies have linked low adiponectin levels to hypertension (191-193). There

remains some uncertainty about the role played by insulin resistance in the adiponectin-hypertension relationship as some studies report an association only among participants with insulin resistance, but at least some evidence indicates that low adiponectin levels may affect the development of hypertension at an early stage, without involvement of insulin resistance (192).

Despite adiponectin's anti-inflammatory and anti-atherogenic properties, evidence of a reduction in risk of CVD in relation to increased adiponectin levels has been conflicting (194). Using a case-control design nested within the largely white Health Professionals Follow-up Study, Pischon and colleagues examined adiponectin levels in 266 males with non-fatal myocardial infarction and fatal coronary heart disease and 532 controls. After multivariate adjustment, the relative risk for CHD (including both fatal and non-fatal events) was 0.56 (95% CI=0.32-0.99) comparing the highest quintile of adiponectin to the lowest quintile (195). A case-control study of coronary artery disease in Japanese men had similar results with an OR of 0.5 for the highest versus lowest quartile of adiponectin (196). A meta-analysis of seven prospective studies conducted through 2005 with nonfatal MI or CHD death as the outcome found an odds ratio of 0.84 (95% CI=0.70-1.01) comparing the top third of adiponectin levels to the bottom third (197). A large population-based longitudinal study in Germany (published after the meta-analysis) found that the hazard ratio for incident coronary heart disease (CHD) was 0.62 (95% CI=0.39-0.98) comparing the highest to the lowest tertile of adiponectin but this association was attenuated after adjustment for HDL-cholesterol level (198). Other recent studies have found no significant association between adiponectin levels and CVD events after adjustment for other risk factors (199, 200). Lara-Castro et al. have speculated that the studies to date indicate that the independent association

between adiponectin and CVD risk is not strong but rather adiponectin may affect CVD risk indirectly via its effects on intermediaries such as HDL cholesterol (190).

The first study to examine whether adiponectin is associated with coronary heart disease among both blacks and whites included 1,044 blacks and 1,429 whites ages 70 to 79 from the Health, Aging, and Body Composition Study (143). This study found that adiponectin was negatively associated with a higher prevalence and incidence of CHD in black subjects but not in white subjects (143). Kanaya et al. have suggested that biological differences that affect the action of adiponectin, such as differences in levels of lipoprotein lipase or the proportion of visceral fat, may be responsible for these racial variations (143). Overall, however, there is a dearth of reports examining cohorts with large numbers of blacks and females in the CVD-adiponectin literature.

Genetic variants in ADIPOQ, ADIPOR1, and ADIPOR2 in relation to obesity

Polymorphisms in ADIPOQ have been examined in relation to a variety of obesity phenotypes in many studies. Nomenclature of the SNPs has evolved over time; in ADIPOQ, two SNPs, rs2241766 (commonly called 45T□G or SNP45) and rs1501299 (commonly called 276G□T or SNP276) were among the earliest to be discovered and most frequently studied in this gene.

SNP rs2241766 is a silent polymorphism in exon 2 of ADIPOQ that results in a T to G substitution and it has been examined in relation to BMI in several studies. Carriers of the G allele in rs2241766 have been observed to have higher BMI values than TT homozygotes in populations from Taiwan (201) and France (202) as well as in a subgroup of Germans with a family history of diabetes (203). However, carriers of the G allele in rs2241766 have also been reported to have lower BMI values than TT homozygotes (204-206) or to be unrelated to BMI (207-209). Waist circumference was also found to be associated with the T to G

substitution in rs2241766 in one study (210). Another SNP that has been frequently examined in relation to obesity is rs1501299 located in intron 2 of ADIPOQ. This SNP was not associated with BMI in several studies (205-209)

Early studies examined SNPs rs2241766 and rs1501299 individually while more recent studies have looked at haplotypes comprised of these two SNPs in relation to body size. In the Nurses Health Study, women with the haplotype defined as T at rs2241766and G at rs1501299 were found to have a higher prevalence of obesity than women with other haplotype structures (204). Similarly, participants in an Italian study with haplotypes including T at rs2241766and G at rs1501299 were found to have higher body weight and higher waist circumference than those with other haplotype structures (211).

Other variants in ADIPOQ have been reported to be associated with obesity although few have been replicated. At least one group examined a SNP in the intronic region between exons 2 and 3 (called IVS2+G62T) in which those with the GT genotype had higher BMI those with the GG genotype (210). A SNP in the proximal promoter region of ADIPOQ (formerly called SNP -11377, now rs266729) has been examined in at least six studies and no association between this SNP alone and BMI was observed in most of the reports (206, 208, 209, 212, 213). In a group of diabetics, those with the CC or CG genotype of rs266729 were found to have higher BMI than those with the GG genotype (214). Another SNP in ADIPOQ (formerly called -11391, now rs17300539) has also been examined in several studies with obesity phenotypes as the outcome. Associations have been inconsistent with some reports having null findings (208, 209), another reporting that the A allele in rs17300539 is associated with lower BMI and waist and hip circumferences (213), and another reporting no

association with BMI or waist circumference but an association with visceral adipose tissue (VAT) as measured by computed tomography (CT) (206).

Only one study has examined polymorphisms in the adiponectin receptors, ADIPOR1 and ADIPOR2, in relation to body size, and there was no association found between any SNPs and obesity-phenotypes including BMI, percentage body fat, or waist circumference (205).

While the evidence is not yet conclusive, genetic polymorphisms in the genes encoding adiponectin and adiponectin receptors do appear to be linked to adiponectin and obesity-related phenotypes. However, despite the known differences in obesity between whites and blacks, single nucleotide polymorphisms (SNPs) in ADIPOQ, ADIPOR1, and ADIPOR2 have been examined almost exclusively in populations that did not include individuals of African descent.

Genetic variants in ADIPOQ, ADIPOR1, and ADIPOR2 in relation to adiponectin levels

Several studies have examined polymorphisms in the gene encoding adiponectin in relation to measured concentrations of adiponectin in blood although most of the early studies were small and few included sizeable numbers of women or blacks. A 2007 meta-analysis was conducted of the studies up to this date examining variants in ADIPOQ in relation to adiponectin levels. Three SNPs met the criteria for meta-analysis (at least 2,000 individuals had been genotyped in all studies combined): rs17300539, rs1501299, and rs2241766. Two of these SNPs (rs17300539, genotyped in five studies, and rs1501299, genotyped in twelve studies) were significantly associated with adiponectin levels in the meta-analysis while rs2241766 (genotyped in ten studies) was not (215). Notably, all of the studies included in this meta-analysis included study populations that were exclusively white or Asian. Not

included in the Menzaghi meta-analysis was a study of 747 Spanish individuals which found that the G/G genotype of rs1501299 was associated with lower adiponectin levels compared to carriers of the G/T or T/T genotype (216) as well as a study of 867 Korean women which found similar results to the Spanish study (217).

As genotyping costs have been reduced and genotyping platforms have been developed to accommodate large numbers of SNPs, research teams have begun genotyping larger numbers of SNPs, often with a tag-SNP approach, and examining them in relation to adiponectin levels. Heid et al. genotyped 15 tag SNPs in 1,770 healthy Austrian adults (N=663 female) and found significant associations with adiponectin levels for 11 of these SNPs individually and with several haplotypes, many more than had previously been detected in other studies (218). In two samples of white women from the United Kingdom (UK), Kyriakou et al. found and replicated positive associations for four SNPs in ADIPOQ (rs17300539, rs182052, rs16861209, and rs1501299) (219). Among 2,543 white adults in the Framingham Offspring Study (half female), 22 tag SNPs in ADIPOQ were genotyped and two were found to be strongly associated with adiponectin levels: rs17300539 (which was also significant in the 2007 meta-analysis, the Austrian study, and the UK studies) and rs822387.

Two recent genome-wide association studies (GWAS) reported a strong association with adiponectin levels and SNP rs17366568 on ADIPOQ in European whites (220, 221). Ling et al. also found two additional SNPs in ADIPOQ to be strongly associated with adiponectin levels (rs3774261 and rs6773957) in European whites (220). Heid et al. found low regulatory potential for rs17366568 indicating that it is likely located near to a functional variant but is not functional itself (221).

To date, only one study (the GWAS of white Europeans by Ling et al.) has examined adiponectin levels in relation to SNPs in ADIPOR1 and ADIPOR2 and no significant associations were found (220).

Summary

Taken collectively, the research conducted to date linking adiponectin and obesity provide compelling evidence that careful examination of this association will help us to better understand the development and prognosis of several high burden diseases including several cancers, type 2 diabetes, and cardiovascular disease. In addition, the lack of studies focused on adiponectin among blacks highlights a clear and immediate need for studies in populations with a sizable proportion of black participants. In particular, few studies have quantified the blood levels of adiponectin in relation to body size in a large population of blacks and whites over a wide range of age and body size in order to evaluate potential differences in the magnitude and form of the association across racial groups. Additionally, while associations with body size, adiponectin levels, and genetic polymorphisms in the ADIPOQ genes have been observed in several populations, few studies have included sufficient numbers of black subjects to assess whether the observed associations are the same across racial groups. Finally, very few studies have examined ADIPOR1 and ADIPOR2 polymorphisms in relation to adiponectin levels and body size and none have been conducted in black participants. Overall there is only limited information in the literature regarding the relationship between adiponectin and environmental and genetic correlates among blacks. The work presented here takes an important step in filling this void in our understanding of these relationships and indicates areas for future research.

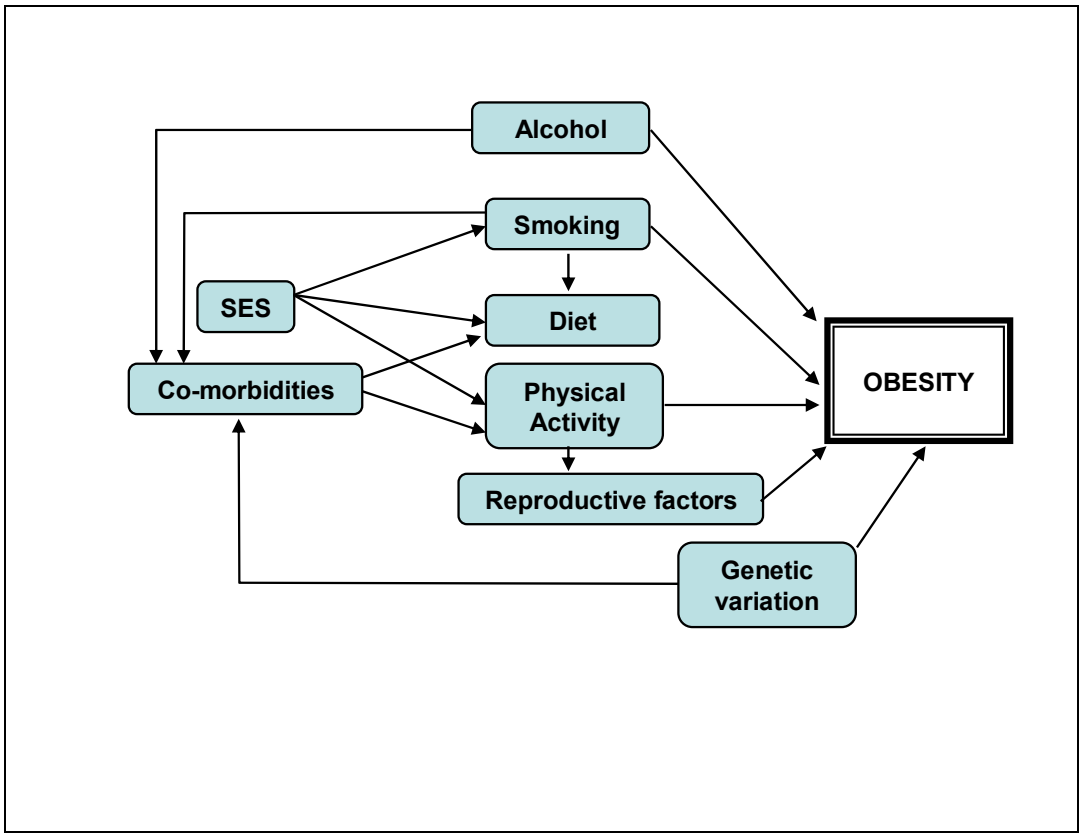

Figure 1.1 Individual-level behavioral and environmental determinants of obesity

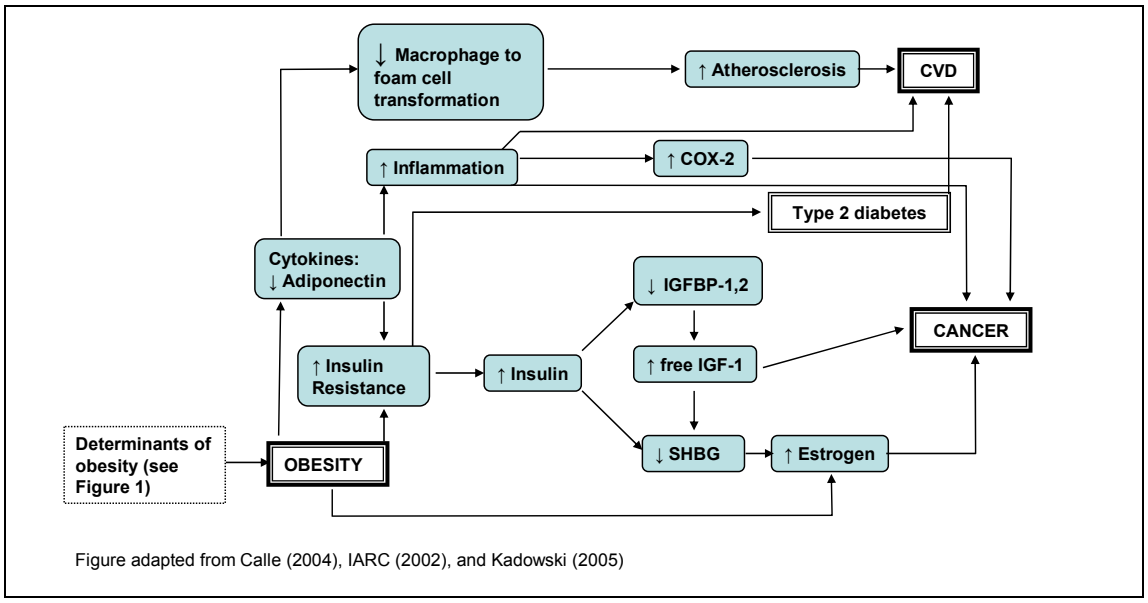

Figure 1.2 Selected obesity-mediated pathways linking cancer and CVD

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CHAPTER 2: RESEARCH METHODS

Overview

I conducted a cross-sectional analysis of a sample of women enrolled in the Southern Community Cohort Study (SCCS) in order to examine associations between adiponectin levels, obesity (as measured by body mass index [BMI]), environmental and behavioral factors, and genetic variants in the ADIPOQ, ADIPOR1, and ADIPOR2 genes. These women were previously selected for a separate study funded by Susan G. Komen for the Cure to examine behavioral and genetic determinants of obesity in women enrolled in the SCCS (grant number OP05-0927-DR1), hereafter called "The Komen Obesity Project". A sample of 1,000 white women and 1,000 black women was utilized for these analyses. Institutional Review Board (IRB) approval was granted for this study by the IRBs at Vanderbilt University, Meharry Medical College, and the University of North Carolina at Chapel Hill. Three specific aims were examined in this work:

Specific Aim 1: Determine whether adiponectin levels among black women as compared to white women are associated with BMI and environmental and behavioral factors including physical activity, energy and nutrient intake, alcohol consumption, smoking, reproductive factors, and co-morbid conditions.

Specific Aim 2: Determine whether adiponectin levels among black women as compared to white women are associated with genetic polymorphisms in tag-SNPs in the ADIPOQ, ADIPOR1 and ADIPOR2 genes and, in exploratory analyses, interactions between environmental factors and genetic polymorphisms in the ADIPOQ, ADIPOR1 and ADIPOR2 genes.

Specific Aim 3: Determine whether BMI among black women as compared to white women are associated with genetic polymorphisms in tag-SNPs in the ADIPOQ, ADIPOR1 and ADIPOR2 genes; and in exploratory analyses, interactions between environmental factors and genetic polymorphisms in the ADIPOQ, ADIPOR1 and ADIPOR2 genes.

Below, details of the Southern Community Cohort Study (the parent study for the proposed research), the study sample utilized for the research, assessment of the outcomes and exposures outlined in Specific Aims 1, 2, and 3, and the statistical methods used in the analyses are described.

Parent Study: The Southern Community Cohort Study

This research project was based on a sample of women selected from the Southern Community Cohort Study (SCCS) in February 2006. The SCCS is a prospective cohort study being conducted by Vanderbilt University in collaboration with Meharry Medical College and the International Epidemiology Institute. The SCCS began participant enrollment in 2002. The objective of the SCCS is to examine a large population of both black and white individuals to seek answers to unresolved and understudied questions regarding the underlying causes of certain cancers, especially causes of the important disparities in cancer incidence and mortality across racial groups (1). Several large cohort studies have been conducted in various geographic areas of the United States, but none include large numbers of both black and white participants or a majority of participants from rural, low-income, or disadvantaged populations. The SCCS is thus unique in its position to investigate factors that underlie disparities in health risks related to race and socioeconomic status.

SCCS participant recruitment

At the time of the selection of the current study sample (February 2006), over 47,000 adults age 40-79 from the southeast region of the United States had been enrolled into the SCCS through community health centers (CHCs) located in both rural and urban locales throughout twelve southeastern states. These CHCs are government-funded health care facilities that provide basic health services primarily to low-income individuals (2). Enrollment of cohort members through CHCs began in April 2002 and more than two-thirds of the SCCS participants who were enrolled through the CHCs were black. The location of the 60 CHCs that enrolled SCCS participants through February 2006 are shown in Figure 2.1.

Potential study participants were CHC patients, friends or family members who were accompanying patients, and users of CHC pharmacies. Participants were recruited to join the study in-person at the CHC by a trained study interviewer. In order to be eligible to join the SCCS, participants must have been between the ages of 40-79, not under treatment for cancer in the past year (with the exception of non-melanoma skin cancer), able to provide an address and telephone number, and English-speaking.

Baseline interview

After obtaining informed consent, a trained study interviewer conducted an in-person baseline interview for each participant which lasted approximately 50 minutes. The interview was conducted using a laptop computer and a specially-designed computer-assisted personal interview (CAPI) with substantial logic-checking and skip pattern features built into the instrument. The interview gathered information including demographics, anthropometric measurements, physical activity, smoking, reproductive history, personal medical history, family medical history, medication use, emotional well-being and social support, religion/spirituality, health insurance, use of medical and cancer screening services,

and occupational history. The longest section of the interview was an 89-item food frequency questionnaire designed specifically for this population. It was empirically derived from the third National Health and Nutrition Examination Survey (NHANES-III) to include foods commonly eaten by Southerners and foods that are likely to differ between blacks and whites (3, 4). Alcohol consumption and use of vitamin supplements were also assessed in the baseline interview.

SCCS Participants

The SCCS was very successful at enrolling the target populations of blacks and people of low socioeconomic status. As of February 2006 (the time at which the sample of women for this research was selected), 61% of the over 56,000 enrolled participants were female and 75% were black. Of the women enrolled through February 2006, 61% reported a yearly household income less than $15,0000 (Table 2.1). Nearly 40% of these women reported not having health insurance. The prevalence of co-morbidities and obesity at baseline was high among the women in the SCCS with 23% reporting a diagnosis of diabetes and 60% reporting a diagnosis of hypertension. A wide range of body sizes was evident in the women enrolled in the SCCS with very stable proportions in each category from healthy weight (BMI between 18.5 and 24.9 kg/m2) to overweight (BMI between 25.0 and 29.9 kg/m2) to obesity classes I, II, and III (BMI 30.0-34.5, 35.0-39.9, and > 40.0 kg/m2, respectively) (4). The distribution of body size is consistent with NHANES data that show that black women tend to be heavier than white women (5) and also with numerous reports that show women of low SES (such as are found in the SCCS) generally have a distribution of BMI that is higher than that of women of high SES (6).

Data quality considerations

The baseline interview of the SCCS was conducted by study interviewers at each of the participating CHCs. Study interviewers from each CHC were trained together by the SCCS study team during intensive one-week training periods. Standardized interviewing techniques such as remaining neutral and using a common set of probes to elicit appropriate information from participants were stressed. Quality control measures were in place to assess the quality of the interviews during the time that the interviewers were working in each CHC. These measures included telephone calls to a random subset of enrolled participants to confirm answers to a selected set of approximately ten interview questions and evaluations of tape recorded interviews by study staff.

Error detection in baseline interview data

Study interviewers conducted the baseline interview using a laptop computer and a computer-assisted personal interview (CAPI). The CAPI included built-in ranges for each variable as well as extensive logic checking and skip patterns. Interview data were transmitted to a central server each evening from the CHCs and a daily quality control report was run to check for any abnormalities. The use of the CAPI helped to ensure high quality, relatively clean data that was consistent within each participant.

For some measures, the large range of acceptable values allowed for some error to be introduced. This is relevant for the self-reported weight and height data which were used to calculate BMI, one of the measures used in this project. For example, women were asked to report their current weight with values between 50 and 500 pounds accepted by the CAPI. Data entry errors within this range could generally not be detected by the CAPI. However, the self-reported data are believed to be generally of high quality for several reasons. First, it is believed that the in-person nature of the interview was a deterrent for gross under- or over-reporting of weight by the participants. Also, for approximately 25% of the SCCS

population, weight and height were measured in the CHC as part of the medical visit on the day of the baseline interview, and this information was abstracted from the medical record by the study interviewer. The correlation is extremely high overall for the BMI values calculated from self-reported height and weight data compared to BMI calculated from the medical record data (Pearson correlation coefficient > 0.95). For the sub-sample of participants with both medical record and self-reported values available, the self-reported height and weight were compared to the medical record-abstracted height and weight and inconsistencies were resolved by consensus of the SCCS study team as to the most plausible correct value. In the rare event that a plausible value could not be determined, the value was set to missing. For the purposes of this project, only women with non-missing self-reported height and weight values were eligible to be sampled.

Blood samples

Participants were also asked to donate a blood sample at the time of the baseline interview in the CHC. If blood was refused, participants were asked to donate a buccal cell sample. Only the 7% of participants reporting prior hepatitis infection (of any type) and the 1% reporting HIV/AIDS were not asked for a blood or buccal cell sample (because of shipping regulations). Nearly all participants (98%) who were asked to donate a biologic specimen (blood or buccal cells) did so. Overall, about half of the cohort members provided a blood sample with the other half choosing to provide a buccal cell sample instead of blood.

Characteristics of women enrolled through February 2006 who did and did not provide blood samples are described in Table 2.2 (women with HIV or hepatitis who were prevented from being eligible to provide a sample are not included); because almost the entire cohort who are eligible to provide a biologic sample did so (98%), the column of women who did not provide a blood sample largely reflects the women who chose to donate

a buccal cell sample rather than blood. Slightly more white women provided a blood sample compared to black women (26% versus 21%). Missing values were also slightly more common for all of the characteristics for women who did not provide a blood sample compared to women who did. Generally, though, the women were similar across groups of blood provision.

The blood sample was drawn in the CHC laboratory by a staff phlebotomist and generally shipped on ice on the same day of collection for next-morning delivery to Vanderbilt University. Once received at Vanderbilt, blood samples were spun at 1,500g for 10 minutes, using a refrigerated centrifuge (at 4°C). The plasma was then removed and transferred to four sterile 2mL cryovials. White blood cells were aliquoted into two 2mL vials and red blood cells were stored in two 2mL vials. For each sample, serum was transferred into four 2mL vials and blood clot into two 3.5mL vial. All samples are stored in freezer boxes and kept at -80°C. A computerized inventory tracking system has been developed, which can quickly track and locate the storage position of all samples for each cohort participant by study ID.

Several safeguards were in place to ensure that errors in the blood collection were kept to a minimum. First, when blood was collected from each participant at the CHC, a pre-printed bar code label was attached to the blood tubes to correspond to the ID number assigned to each participant by the CAPI. Daily tracking of blood samples and ID numbers was conducted to ensure that the labeling was done properly. The time and date of the sample collection was recorded on the tube of blood and was entered into an Access database at the Vanderbilt laboratory. Quality control reports were run each day to check for data entry errors in the time and date fields.

SCCS Biospecimen Pilot Study

In 2005, after three years of enrollment into the cohort, 792 participants were selected for the SCCS Biospecimen Pilot Study. The goal of the SCCS Biospecimen Pilot Study was to establish the feasibility and improve the efficiency of utilization of the SCCS biospecimen repository (including blood, buccal, and urine samples) for future examinations of genomic, proteomic, hormonal and other potential markers of cancer and other disease risk.

The sample of 792 was drawn using a 2 x 2 x 3 x 3 factorial design based on four factors which included sex (male/female), race (black/ white), smoking status (current/former/ never), and BMI (<25, 25-29, >30 kg/m2). Eight separate projects were undertaken involving blood samples from these 792 participants with including an investigation of blood biomarkers for obesity which measured serum adiponectin and leptin levels. The Hormone Assay Core Laboratory in the Diabetes Research and Training Center at Vanderbilt University, funded by the National Institute of Diabetes, Digestive and Kidney Diseases successfully measured adiponectin levels from thawed serum in the pilot study participants.

Race-specific measures of adiponectin for the women in this pilot study are presented in Table 2.3. Adiponectin levels were successfully measured in 196 of 198 white women and 195 of 198 black women (total N=391). Adiponectin levels were significantly higher among white women than among black women (two-sided t-test p-value <0.0001). This difference across races is consistent with observations from other studies comparing adiponectin levels between whites and blacks (7-14). The adiponectin measure was highly variable in this pilot study with standard deviations of 12.4 and 19.1 for black and white women, respectively. Among the women in the biosample project, the coefficient of variation (CV) for within-subject measures was 7.8% for the adiponectin assay.

Sample selection

This project utilized the sample of women already selected for The Komen Obesity Project which included several separate studies under a large grant from Susan G. Komen for the Cure to examine behavioral and genetic determinants of obesity in women enrolled in the SCCS. The sample was selected first by including the 395 women who were previously included in the SCCS Pilot Biomarker Project (after excluding one women who withdrew from the SCCS), and second, by selecting a random sample of 1,605 women from the SCCS population. The sample of 1,605 women was chosen from an initial pool of 28,158 women enrolled in the SCCS as of February 26, 2006. Because the Komen Obesity Project included the measurement of blood biomarkers as well as genetic markers, only women who provided a blood sample (not a buccal cell sample) were eligible for selection. Thus, of the 28,158 women initially identified, 14,093 were eligible to be sampled based on the availability of a blood sample. The 14,093 women were flagged as ineligible for selection for several reasons (and more than one may have applied to any woman). Women were excluded if they donated the blood sample on a different day from their baseline interview (N=288); donated a sample that was damaged during shipping or processing (N=76); reported a racial/ethnic background other than white or black (N=772); had a BMI outside the range of 18.5-45 kg/m2 (N=2,459); were missing data on questions relevant to menopausal status, blood collection, or dietary intake (N=1,195); reported a prior diagnosis with breast cancer (N=166); or were included in the SCCS pilot biomarker project (N=395), leaving a population of 10,585 women from which to select the 1,605 women.

Sampling was based on three stratification factors including two strata of race (white or black), two strata of menopausal status (pre- or post-), and four strata of BMI (18.5-24.9, 25-29.9, 30-34.9, and 35-45 kg/m2). The long-term goal of the Komen Obesity Project was

to further our understanding of obesity as a risk factor for breast cancer and thus the sample was stratified on menopausal status because the obesity-breast cancer relationship may differ before and after menopause (15). BMI stratification was utilized in order to ensure adequate numbers of women across all levels of body size.

These sixteen strata (2x2x4) of race-menopausal status-BMI were first populated by the 395 women from the pilot biomarker project and then by random selection of the 10,585 eligible women. A total of 127 women were selected for each stratum. This included 125 women as the initial sample and two additional women who served as alternates in the event that unforeseen problems arose in the lab with the blood sample for the women selected initially.

Thus, the final sample used in these analyses included 2,000 women, stratified by race (white/black), menopausal status (pre-/post-), and BMI (18.5-24.9, 25-29.9, 30-34.9, and 35-45 kg/m2).

Table 2.4 shows descriptive characteristics of the women who provided a blood sample (N=14,093), were eligible for selection into the proposed research after exclusionary criteria were applied (N=10,585), and were selected for the final sample (N=2,000). These tabulations show that the women who were eligible after all exclusionary criteria were applied (N=10,858) as well as the final sample (N=2,000) were very similar to the entire cohort of women with available blood samples in their household income and educational attainment. The age distribution of the final sample of 2,000 women was lower than that of the overall cohort of SCCS women due largely to the stratification of the selected women by menopausal status. By selecting the sample of 2,000 to include 50% premenopausal women, the average age of the sample is lower than that in the overall cohort of women.

Outcome assessment

Adiponectin levels

A single one milliliter aliquot of serum was thawed for each participant and further aliquoted into 50 uL tubes which were used for multiple assays as part of the Komen Obesity Project. Blood levels of adiponectin were measured in serum blood samples and used as an outcome measure for Specific Aims 1 and 2 in this study.

Serum adiponectin levels was determined by immunoassay using the LINCOplex kit (Luminex® xMAPTM Technology, St. Louis, MO) at the Vanderbilt Hormone Assay & Analytical Services Core Laboratory. Samples were assayed in batches of 16 as follows: A total of 125 batches were created with 16 women per batch. Each batch contained one woman from each stratum of BMI-race-menopausal status. Duplicate samples for five randomly selected participants were selected to be included in random batches. In addition, five repeat samples from a pooled serum sample were included randomly in order to assess the reliability and validity of the assay. Each sample was run in duplicate by the laboratory resulting in two measures of adiponectin for each selected sample (and up to four measures for the five women randomly selected as duplicates). The coefficient of variation (CV) was calculated for each sample as the standard deviation of the two measures divided by the mean of the two measures. Adiponectin levels were successfully measured in 1,992 of the 2,000 samples (eight samples failed due to a filter plate error or low sample volume). The intra-assay coefficient of variation was 9.4% overall and was similar to other studies that have used this assay and reported CVs (CV range 1.8% to 13.3%) (7, 8, 13).

Body mass index

Body mass index (BMI) was the primary outcome for Specific Aim 3. BMI was also used as an exposure measure in Specific Aim 1. During the baseline SCCS interview, participants

were asked for their current weight (or weight before pregnancy if they were pregnant at the time of the interview) in pounds as well as their current height in feet and inches. Weight was converted to kilograms, height was converted to meters, and then BMI was calculated from these self-reported values as [weight (kg)] / [height (m)]2.

The range of BMI values used in these analyses was 18.5 to 45.0 kg/m2 by design. Women were only eligible to be selected for the study sample if they had a non-missing BMI value that was between 18.5 and 45 kg/m2. The proportion of women with missing BMI values was very small (N=393 or 1.4% of the study population). A larger proportion of women (N=2,066 or 7.3%) were excluded based on very low (<18.5 kg/m2) or high (>45 kg/m2) BMI values. These exclusions were put in place in order to have adequate numbers of women across the range of BMI values to reduce model fit problems in the analysis related to sparse data. This range reflects the WHO categories from healthy weight all the way through obesity class III. BMI is one of many measures of body size that can be used to determine whether an individual is overweight or obese. BMI was selected over other body size variables such as waist-to-hip ratio, skinfold measures, or body scans (such as DEXA or CT) due to time and cost constraints associated with the large sample size of the SCCS and the many locations in which participants were enrolled.

The biggest source of error for the BMI measurement came from the use of self-reported weight and height values. Reports in the literature indicate that heavier women are more likely to under-report their weight and over-report their height leading to underestimates of their true BMI (16). We were able to assess the extent of the misclassification via measured height and weight data that were abstracted from CHC medical records for approximately one-quarter of the SCCS participants. BMI values

calculated from self-reported height and weight in the SCCS were very highly correlated with BMI values calculated from medical record data overall (Pearson correlation coefficient > 0.95) as well as within strata of race, education, income, and BMI. It is also expected that the nature of the in-person interview reduced gross under- or over-reporting by participants that may occur more often in mailed or telephone questionnaires.

Exposure assessment: Environmental and behavioral factors

Potential environmental and behavioral determinants of adiponectin levels were obtained from the baseline SCCS interview. They included demographic factors as well as lifestyle factors such as physical activity, reproductive factors, total energy intake and specific nutrient intakes, alcohol consumption, smoking, and co-morbid conditions.

Age. Age at the time of the baseline interview was calculated as the difference in the date of baseline interview and the reported date of birth. Age was generally treated as a continuous measure.

Income. Annual household income was reported in the baseline interview (for the year prior to the interview) in broad categories including <$15,000, $15-24,999, $25-49,999, and >$50,000-$99,999, and $100,000+. Because of the small number of participants who reported income in the highest category, the top two categories were collapsed into $50,000+.

Education level. Educational attainment was also reported by the participants in the baseline interview. For stability of estimates, education was grouped into categories for analysis including <9 years, 9-11 years, 12 years or completion of high school, and some college or more.

Physical Activity. Summary physical activity measures were calculated from questions asked in the SCCS baseline physical activity questionnaire. This instrument was developed for the SCCS and was designed to comprehensively assess both active and sedentary

behaviors done at home, work, and during leisure-time. Five questions about sedentary behaviors asked for the amount of time per day spent sitting in a car or bus, at work, watching TV or seeing movies, using a computer at home, and for other reasons (examples provided were sitting at meals, talking on the phone, reading, playing games, or sewing). Time spent in light, moderate, and strenuous activity at home and work were assessed separately for weekdays and weekends and then combined using weighted averages. During the interview, handcards were given to the participants with examples of light work (examples were standing at work, light office work, shopping, cooking, child care), moderate work (examples were manufacturing work cleaning house, gardening, mowing lawn, home repair), and strenuous work (examples were loading trucks, construction work, farming). Two questions eliciting time spent in moderate sports (examples were bowling, dancing, golf, or softball) and vigorous sports (examples were jogging, aerobics, bicycling, tennis, swimming, weight lifting, or basketball) were also asked. Two walking questions asked about time spent walking slowly (examples were moving around the house, walking at work, walking the dog) and walking fast (examples were climbing stairs, walking for transportation, or for exercise). All of the physical activity behaviors described above were assessed at the time of the baseline SCCS Interview. The same examples were provided to all participants, regardless of gender or race. The SCCS study team has developed algorithms for transforming self-reported hours and minutes of physical activity into summary quantitative measures of hours per day or metabolic equivalent (MET)-hours per day based on the MET values suggested in the Compendium of Physical Activities by Ainsworth and colleagues (17). Total activity MET-hours per day includes light, moderate, and strenuous household and occupational work as well as moderate and vigorous sports.

Total physical activity (MET-hours/day) was evaluated in quartiles based on the distribution of the entire sample of 2,000 women.

Dietary Intake. Total energy intake as well as specific macronutrient intakes (including fat, carbohydrate, and protein) was calculated from the 89-item food frequency questionnaire (FFQ) included in the baseline interview. The SCCS investigators have developed a computer program to score the FFQ based on sex-, age-, and region-specific food intakes from 24-hour dietary recalls conducted by NHANES and CSFII (4). The program outputs raw energy intake, raw nutrient intakes for individual macro- and micro-nutrients, energy-adjusted nutrient intakes, and dietary intake using food group servings. For the current study, total energy intake as well as macronutrient intake (fats, carbohydrates, and proteins) and fiber intake were examined in relation to adiponectin levels and obesity. Total energy as well as the three macronutrients and fiber were selected because of their known or suspected associations with obesity and because of the dearth of information available on their association with adiponectin levels. Women were ranked based on quartiles of nutrient intakes based on the distribution of the entire sample of 2,000 women. While the absolute energy intakes estimated from an FFQ are not adequately measured, the ranking of women based on quartiles was appropriate for FFQ-derived intakes and has been shown to be a valid measure of dietary intake in epidemiologic studies with a variety of disease outcomes (18).

Alcohol consumption. Participants were asked about the frequency and amount of alcohol consumption in the past year during the SCCS baseline interview. Light beer, regular beer, red wine, white wine, and liquor were assessed separately from each other. All alcohol questions (light beer, regular beer, white wine, red wine, and liquor) related to frequency were asked “Over the past year, how often did you drink [Beer/Wine/Liquor]?” and

respondents were given the answer choices of Never, Rarely, Once per month, 2-3 times per month, once per week, 2-3 times per week, 4-6 times per week, once per day, or more than once per day. Participants were also asked how many beers, glasses of wine, or number of liquor drinks they had on a typical occasion. Servings per day were calculated from the frequency measures (for example, once per week will be weighted as 1/7 drinks per day), then multiplied by the quantity reported and finally combined into a single summary measure of number of alcoholic drinks per day. Alcohol consumption was categorized into 0, 1- < 2, and 2+ drinks per day for analysis.

Smoking. In the SCCS CAPI, participants were first asked whether they had smoked more than 100 cigarettes in their lifetime. If they answered 'yes', they were asked if they smoked cigarettes currently, at what age they started smoking, and the average number of cigarettes they smoked per day (at the time of the baseline interview). If they were not current smokers, participants were asked for the age at which they quit smoking cigarettes. From these CAPI questions, women were categorized as never smokers (answered 'No' to smoking more than 100 cigarettes in their lifetime), former smokers (answered 'Yes' to smoking more than 100 cigarettes in their lifetime but 'No' to smoking cigarettes now), or current smokers (answered 'Yes' to smoking more than 100 cigarettes in their lifetime and 'Yes' to smoking cigarettes now). These categories were treated as a single categorical variable with three levels. Alternatively, duration of smoking was also calculated as none for never smokers and in number of years (by subtracting age at starting from age at quitting). Smoking intensity was calculated by multiplying the amount by the duration of smoking (assuming that the current number of cigarettes smoked per day reflected past smoking history). These three alternative measures of smoking were examined and as expected, were found to be highly

correlated with one another. Thus, the measure of smoking with the highest univariate association with adiponectin was selected for examination in the multivariate models. This measure was smoking duration which was categorized as never smoker, <20, 20-29, and 30+ years.

Menopausal status. Menopausal status was assessed in the SCCS baseline interview by asking women "Have you ever been through menopause, or have your menstrual periods stopped for at least six months?" Women who answered 'Yes' to this question were classified as post-menopausal while women who answered 'No' were classified as pre-menopausal.

Reproductive factors. Factors related to pregnancy and breastfeeding were obtained from the SCCS baseline interview. Participants were asked "How many of your pregnancies have resulted in a live birth?" From this question, the number of live births will be categorized into none, 1-2, 3-4, and 5+. Age at menarche (in years) was examined in categories of ages <12, 12, 13, 14, and 15+. Number of months of breastfeeding was highly correlated with the number of live births but was correlated less strongly with adiponectin in univariate analysis compared to number of live births so breastfeeding was dropped from the multivariate analyses.

Co-morbid conditions. SCCS participants were asked if they had ever been told by a physician that they had a variety of co-morbid conditions including diabetes, heart attack or coronary artery bypass surgery, hypertension, high cholesterol, and depression. Each of these conditions was examined as a dichotomous variable (Yes/No).

HDL cholesterol. Using blood samples collected at baseline enrollment into the SCCS, high-density lipoprotein (HDL) cholesterol was measured in serum by the Vanderbilt Lipid

Laboratory using the ACE Clinical Chemistry System and the ACE HDLC Reagent (#SA1038) following the manufacturer's protocols (Alfa Wassermann, Inc, West Caldwell, NJ). The intra-assay coefficient of variation was 1.6%. While the samples in which cholesterol was measured were convenience and not fasting samples, HDL-cholesterol was not associated with fasting status (p=0.5 for blacks, p=0.4 for whites comparing fasting versus non-fasting levels) (Table 2.5).

Exposure Assessment: Genetic markers

Selection of SNPs.

For specific Aims 2 and 3, genetic variation in the ADIPOQ, ADIPOR1 and ADIPOR2 genes were considered to be the primary exposure measures. SNP selection included the selection of variants that represent common variation in the genes of interest via tag SNPs. The International HapMap project was the primary data source for the selection of the SNPs (19). The HapMap Project was begun in 2002 to provide a public resource to support medical genetic research (19). Phase I of the project sought to genotype at least one common SNP every 5 kilobases across the human genome using four geographically diverse populations (20). 269 samples were genotyped including 30 parent-offspring trios from Yorubans in Ibadan, Nigeria (abbreviated as the YRI population); 30 parent-offspring trios from Utah in the United States (abbreviated as the CEU population); 45 Han Chinese in Bejing, China; and 44 Japanese in Tokyo, Japan (20). In 2005, Phase I was complete and the International HapMap Consortium reported that over 1,000,000 SNPs had been identified, examined for quality control purposes, and found to be polymorphic in the 269 samples (20). Phase II of the HapMap Project was completed and reported upon in 2007; it included the genotyping of an additional 2.1 million SNPs in the same 269 samples to obtain a SNP

density of approximately 1 per kilobase (21). The HapMap Project has been widely used as a resource for the selection of tag SNPs across the genome (21).

The pattern of linkage disequilibrium (LD), a measure of dependence between two genetic loci, varies a great deal from population to population. This variation is due to factors such as genetic drift, migration, admixture, and rapid population expansion (22). For example, in a population with fewer generations (i.e. archeologically young), there is likely to be higher LD because there have been fewer recombination events compared to a more ancient population (with many more generations since its founding) in which it may be more difficult to detect LD. This variation in LD between populations has important implications for the search for disease predisposing genes. Important for this project which included both white and black women is the implication that different numbers of SNPs for the same gene may be required for each population in order to capture the variation within a given region. It has been shown that there is less LD in African populations than in Western European populations which indicates more variation in allele frequency and thus that more SNPs may need to be genotyped in an African population that in a Western European population to detect associations between markers and diseases (20). Reich et al. have suggested that the presence of large blocks of LD in northern European populations compared to smaller blacks of LD among Africans suggests that the resolution of gene maps may be much finer in the populations with smaller blocks of LD, thus resulting in a better ability to pinpoint specific SNPs responsible for disease in these populations (23).

Using the Tagger pairwise method implemented in Haploview, tag SNPs were selected based on a minor allele frequency greater than 5% in either the CEU or YRI populations and an r2 value >0.8 (where r2 is a measure of the correlation between alleles at

two markers) as determined from the HapMap Project (19). Because the Illumina GoldenGateTM assay was selected to be the genotyping platform for this project, the selected tag SNPs were evaluated by Illumina, and tag SNPs were reselected when Illumina indicated that a particular SNP was not likely to succeed using GoldenGateTM.

The SNPs that were genotyped in ADIPOQ are listed in Table 2.6 and are diagramed in Figure 2.2. A total of 25 SNPs were selected to provide adequate gene coverage for both the white and black women included in this study. The SNPs that were genotyped in ADIPOR1 are found in Table 2.7. Nineteen SNPs were selected for this gene. A list of SNPs genotyped in ADIPOR2 is found in Table 2.8. A total of 28 SNPs were selected to provide coverage of this gene. Figures 2.3 and 2.4 show the gene structure and SNP coverage for ADIPOR1 and ADIPOR2, respectively.

Population stratification

In recent years, an extensive body of literature has been published documenting potential biases in observations from genetic association studies (such as this project) that result from population stratification. Population stratification refers to a particular type of confounding in which substructures that exist within human populations due to non-random mating confound the association between a genetic variant and disease outcome (24, 25). In order for population stratification to create bias in observed measures of association, the gene under study must show variation in allele frequency across subpopulations, and these subpopulations must also have differing baseline risk of the disease outcome (26).

There is clear debate in the literature about the extent of the problem of population stratification (25, 27). Thomas and Witte argue that allele frequencies of many genes vary greatly across populations and that the degree of variation is directly related to the genetic distance between the populations (27). These authors also point to marked differences in

disease rates across populations, and conclude that these two factors are sufficiently important to warrant attention to population stratification in association studies of unrelated individuals (27). Thomas and Witte emphasize the use of related controls for genetic association studies to account for population stratification, which is, unfortunately, a difficult and impractical standard to meet for most studies in terms of recruitment, sample size, and other issues of practicality and efficiency. In a counterpoint piece to the opinions voiced by Thomas and Witte, Wacholder and colleagues state that they believe population stratification is not a serious threat to the validity of well-designed and well-analyzed association studies, at least for cancer outcomes and in non-Hispanics of European descent (25). Wacholder et al. argue that meaningful population stratification is unlikely when multiple ethnic groups are studied unless both the range of disease rates and the frequency of the disease-causing genotype vary greatly (25). Whether their arguments hold in studies of blacks and for such complex disease outcomes as obesity, such as in this project, is unclear. Rebbeck and Sankar (26) strike a balance in their 2005 commentary by claiming that while the evidence is limited to show that population stratification cause biases in most common epidemiologic studies, the potential for confounding is real and researchers should use appropriate study designs or analytic methods to evaluate and account for any bias.

There are several ways to account for population stratification. Beyond using family-based designs (which are costly and often impractical) or simple adjustment for self-reported ethnicity (which may not fully capture the scope of the problem), several different methods have been proposed to address population stratification. Pritchard and Rosenberg suggested an approach known as structured association (SA) (28-30). SA assumes that a heterogeneous study population is composed of several homogeneous subpopulations. Individuals are

assigned to subpopulation clusters based on derived vectors of information regarding ancestral origin using a Bayesian clustering approach. These clusters representing ancestry can be used as covariates in statistical models. Using readily available software packages makes the implementation of SA relatively easy and straightforward. However, the number of subpopulation clusters is user-defined and results may vary based on the number of clusters used. Devlin et al. proposed an alternative approach that they call genomic control (GC) (31). Because population stratification results in over-dispersion of test statistics used to assess association, GC is used to estimate the degree of over-dispersion by testing multiple polymorphisms throughout the genome and then determining the appropriate empirical distribution for a test statistic (24, 31). Price et al. note that GC adjusts test statistics by a uniform factor which may be insufficient for markers that differ strongly across ancestral populations or may result in a loss of power for markers that do not exhibit strong differentiation (32). Price et al. proposed a third method for accounting for population stratification via the use of principal components. The principal components method infers continuous axes of genetic variation from a set of genetic markers, and these components are then used as covariates in the analysis of the genotype-disease association of interest in order to account for population stratification (32).

Selection of Ancestry Information Markers (AIM) SNPs

Freedman et al. demonstrated that genotyping only a few dozen markers cannot rule out modest levels of population stratification and that larger numbers of markers need to be evaluated in order to bring the 95% upper bound on stratification within 10% of the true value (33). Because this project was conducted as part of the larger Komen Obesity Project which utilized a multiplex assay, several hundred AIMs were selected for genotyping and the

resulting genotypes were utilized in these analyses to assess and control for population stratification.

292 Ancestry Informative Markers (AIMs) SNPs were ultimately selected for this project. A list of potential AIM SNPs was generated from two sources. A group at Vanderbilt University led by Dr. Scott Williams generated a list of 360 SNPs that was derived from analyses comparing frequencies between individuals of European (CEU) and African (YRI) descent in HapMap using chi-square values to rank the markers. Additionally, a list of AIM SNPs from an Illumina-designed panel for ancestry estimation was obtained (N=1,508). Allele frequencies for these 1,868 SNPS were updated using HapMap release 22 data. Potential AIM SNPs on the Y chromosome were excluded (because the genotyped sample included only females) as were SNPs without detailed information about chromosome position (N=1826 remaining). The position of each candidate gene in the Komen Obesity Project was identified with 5 Mb flanking on either side. All potential AIM SNPs within any of the candidate genes or the 5 Mb flanking regions were excluded (N=635 remaining). After exclusion of potential AIM SNPs by chromosomal location, additional potential AIM SNPs were deleted that had a minor allele frequency of < 0.05 in both the CEU and YRI populations in HapMap (N=367). Finally, 300 of the remaining 367 potential AIM SNPs were selected based on the highest allele frequency differences between the CEU and YRI populations in HapMap. Of these 300 AIM SNPs, 292 passed the Illumina Scoring algorithm and were sent for genotyping.

DNA Extraction

Genomic DNA was obtained from buffy coat using Qiagen's DNA Purification kits (Qiagen Inc., Valencia CA) following the manufacturer's protocol. The average DNA yield for each sample was about 20 □g, which allowed for numerous PCR amplifications.

Genotyping

The genotyping for this project was conducted in conjunction with the Komen Obesity Project which in total examined 40 candidate genes related to obesity. Genotyping took place at Vanderbilt University in the laboratory of Dr. Jeffrey R. Smith. The genotyping was conducted using the Illumina GoldenGateTM assay (Illumina, Inc. San Diego, CA) (34, 35). A total of 1,536 SNPs (including those located on the three genes of interest for this research) were assayed together using GoldenGateTM. The Illumina assays have very high genotype call rates (99.97% for the 1,536 loci multiplex) and high reproducibility (>99. 9%). Statistical tools within BeadStudio software from Illumina were utilized by Dr. Smith's lab to evaluate genotyping success and confidence. SNPs were excluded with call rates < 85% and samples that were found to be duplicates were removed. Call rates were re-estimated and 1,420 SNPs with call rates > 95% were included in the final analytic dataset for the overall Komen Obesity Project. Blinded QC samples (N=29) and another 171 pairs of duplicated samples were included and the consistency rate was 99.9%. All samples showed a call rate > 90% and thus no samples were excluded based on low call rate.

For this study, all SNPs that were selected in the three genes of interest (ADIPOQ, ADIPOR1, and ADIPOR2) were successfully genotyped and had call rates greater than 95% except for one, rs11612383 located on ADIPOR2 which had a very low call rate and was excluded from further analyses. Of the 292 AIM SNPs that were sent for genotyping, 276 (95%) were successfully genotyped with call rates greater than 95%.

Of the 2,000 women selected for analysis, genotyping was successful for 1,990 (N=996 black and N=994 white women). Twenty-three women were excluded from the analyses because of discrepancies between self-reported race and ancestry estimates derived from STRUCTURE (Figure 2.5). Among women who self-reported their race as 'Black/African

American', six were excluded who had an estimated percentage of African ancestry less than 20%. Similarly, for women who self-reported their race as 'White', 17 were excluded who had an estimated percentage of African ancestry greater than 30%. Overall,1,967 women remained for analyses (N=990 black and N=977 white).

Quality control of genotyping data

Every effort was made to minimize potential errors during genotyping. The laboratory staff remained blinded as to any characteristics of the women selected for the study. Suitable negative and positive controls were run including water samples in random wells and a standard set of control DNA samples. In addition, ten blinded duplicate samples for women in this study sample were genotyped for comparison purposes.

Once genotyping was complete, each allele was assessed for Hardy-Weinberg equilibrium (HWE). The proportion of observed alleles was compared to the expected proportion of alleles using the relationship $p2 + 2pq + q2 = 1$ (where p and q represent the frequencies of each of the two alleles for a given SNP) (36). A chi-square test with one degree of freedom was used to compare the observed and expected proportions and HWE was determined to be violated if the p-value is less than 0.05.

HWE was examined separately for blacks and whites. A total of 12 SNPs had HWE p-values <0.05 and these SNPs were carefully examined for genotyping errors by Dr. Smith's laboratory staff. Because HWE holds only under strict conditions (including random mating, no selection or migration into or out of the population, no mutation, no population stratification, and an infinite sample size) (36) and it is plausible that these conditions were not fully met in this study population, SNPs that were found to be not in HWE but did not appear to have been genotyped incorrectly were decided a priori not to be removed from the analysis. Based on this guideline, only one SNP (rs1648707 located on ADIPOQ) which

deviated extremely from HWE (p=8.6x10-16 in whites and p=1.3x10-16 in blacks) was excluded from further analysis.

Statistical Methods

The statistical methods for this research included univariate data analysis, bivariate data analysis including each exposure and outcome pair, and regression modeling for each exposure and outcome pair with careful consideration paid to confounding, multiple testing, and diagnostics. In addition, the AIM SNPs were used to derive both allelic ancestry clusters and principal components to be used for adjustment for population stratification. Because one of the main interests of this project was to examine differences between white and black women, analyses were stratified by race unless otherwise specified.

Derive ancestry allelic clusters and principal components from Ancestry Informative Markers

The 292 genotyped ancestry informative markers (AIMs) were used to derive two independent sets of variables to be considered for inclusion in the final statistical models to account for population stratification. The first method to be considered was principal components that empirically estimate ancestry based on the method of Price et al. (32). The EIGENSTRAT stratification correction method as implemented in EIGENSOFT version 2.0 software, available from David Reich's laboratory at The Harvard Genetics Department and the Broad Institute, was utilized to derive the first five principal components for each race group (37). The second method under consideration included the derivation of ancestry allelic clusters from STRUCTURE Version 2.2.3 software (http://pritch.bsd.uchicago.edu/structure.html) (29). STRUCTURE infers individual proportions of ancestry from K clusters, where K is specified in advance and corresponds to the number of posited ancestral populations. Individuals are assigned admixture estimates

(i.e., ancestry allelic clusters, or AAC) from multiple ancestral populations, with the admixture estimates summing to 1 across these population clusters. Given that the participants in this project were selected for inclusion based on self-reported race being only white or black, and the number of Asian and Hispanic participants in the SCCS is very low, K was specified to be 2 and thus two AACs were generated for each individual: one for African ancestry and one for European ancestry. AAC proportions (ranging from 0.00 to 1.00) for each individual were estimated from the AIM SNPs.

Both the ancestry allelic clusters derived from STRUCTURE and the principal components partitioning ancestry that were created as output from EIGENSOFT were examined as potential confounders in the regression modeling. Both methods of adjustment for population stratification resulted in nearly identical effect estimates between the SNPs and the outcomes of interest, adiponectin level (Tables A.1a, A.1b, and A.1c) and BMI (Tables A.1a, A.1b, and A.1c). Thus, the STRUCTURE approach (using ancestry allelic clusters) was selected for the final analyses because fewer degrees of freedom were required for the single term for African ancestry compared to the principal components approach.

Univariate data analysis

Frequencies for all categorical exposure, outcome, and potentially confounding variables were computed among the white and black women and examined for outliers. These measures included age, education, and income, physical activity, reproductive factors, total energy intake and specific nutrient intakes, alcohol consumption, smoking, and co-morbid conditions as well as the individual genotypes. Descriptive statistics including means, standard deviations, medians, and ranges were calculated for the continuous measures of adiponectin level and BMI.

Bivariate data analysis

Further exploratory data analysis was conducted via bivariate data analysis of each exposure and outcome pair. The purpose of the bivariate analyses was to better understand the crude associations observed in these data as well as to examine the potential for confounding.

For Specific Aim 1, correlation coefficients were examined between adiponectin level (outcome) and BMI (exposure). Descriptive statistics for adiponectin levels and BMI by categories of environmental and behavioral factors were also examined.

For Specific Aim 2, descriptive statistics for adiponectin levels were tabulated by genotype for each SNP in ADIPOQ, ADIPOR1 and ADIPOR2 (Tables A.3, A.4, and A.5).

For Specific Aim 3, descriptive statistics for BMI were tabulated by genotype for each SNP in ADIPOQ, ADIPOR1 and ADIPOR2 (Tables A.6, A.7, and A.8).

Regression modeling

SAS/STAT software, Version 9.2 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

There were two main objectives in Specific Aim 1: the first was to characterize the relationship between adiponectin and BMI within each race group and the second was to determine predictors of adiponectin after adjustment for BMI. Linear regression models were developed to address each of these objectives. Adiponectin was the outcome variable for these regression models and was treated as a continuous measure. The distribution of adiponectin was skewed and thus, a log-transformation was applied to better meet modeling assumptions (38).

To address the first objective of Specific Aim 1, continuous BMI was treated as the main exposure variable and was regressed on adiponectin. Potential confounders were determined from the literature (as described in Chapter 1) and included age at baseline SCCS

interview, household income, educational attainment, physical activity, HDL-cholesterol, dietary intake (total energy, fat, carbohydrate, protein, and fiber), cigarette smoking, menopausal status, number of live births, age at menarche, and co-morbidities (diabetes, heart attack or coronary artery bypass surgery, hypertension, high cholesterol, and depression). Potential confounders were added to the regression model using backwards model selection with a change-in-estimate criterion of > 5% used to determine confounding (Tables A.9 and A.10). Potential modifiers of the BMI-adiponectin relationship were assessed using the likelihood ratio test (LRT) to compare models with and without interaction terms for BMI and potential modifiers. Using the LSMEANS option in SAS/STAT PROC GLM, adjusted geometric means for adiponectin were calculated from linear regression models that included standard categories of BMI (<18.5, 18.5-24.9, 25-29.9, 30-34.9, 35-39.9, and 40-45 kg/m2) and the final set of confounders.

To address the second objective of Specific Aim 1, a prediction model for adiponectin was developed with log-adiponectin as the outcome. BMI was forced into the model and then environmental and behavioral factors were added sequentially. BMI was forced into the model in order to determine the relative predictive ability of the environmental and behavioral factors after adjustment for obesity which is known to be strongly associated with adiponectin level. With an emphasis on prediction of adiponectin levels, all covariates that appreciably improved prediction of adiponectin were retained in the final model. Improvement in model prediction was assessed using Akaike's information criterion (AIC) which takes into account improvements in the model goodness-of-fit while imposing a penalty for an increasing number of parameters being estimated. Potential predictors were

included in the final model as long as their addition resulted in an AIC value at least one unit lower than the AIC for the smaller-order model (Tables A.11 and A.12).

Standard regression diagnostics were applied to the final linear regression models to ensure that the assumptions of the linear regression model are not violated. These included transformation of adiponectin using a log-transformation; examining the mean, variance, and independence of the residuals from the regression models; examining whether particular observations controlled the fit of the model; and examining whether there was collinearity between any of the covariates in the model (38).

Additional modeling was performed by re-running the final prediction model developed for black and white women separately after stratifying women according to their decade of birth in order to assess potential cohort effects. The strata included 1924-1939, 1940-1949, 1950-1959, and 1960-1969. The results of these models are shown in Table A.13.

For Specific Aims 2 and 3, the general approach to estimating the associations between adiponectin levels (Specific Aim 2) and BMI (Specific Aim 3) and genetic polymorphisms in ADIPOQ, ADIPOR1 and ADIPOR2 was to use linear regression models to examine single SNP-outcome associations (See Tables A.14, A.15, and A.16 for adiponectin as the outcome and Tables A.17, A.18, and A.19 for BMI as the outcome). The genotype frequencies of each SNP were examined separately for black and white women and SNPs with a MAF < 0.01 for a particular race group were not examined further within that race group.

Each of the genotyped SNPs from the three genes of interest was examined individually in relation to adiponectin or BMI using the general (co-dominant) genetic model

of inheritance. The referent genotype was selected to be the most common race-specific homozygous genotype within each race group. For SNPs in which less than 10 women had the rare homozygous genotype, a dominant model was used that combined women with the rare homozygous genotype with those with the heterozygous genotype. The co-dominant genetic model does not impose restrictions on the relationship between the estimates for each genotype. Because there was no a priori hypothesis about the model form for the SNP-adiponectin associations or the SNP-BMI associations, the use of the two degree of freedom test for the co-dominant model was used because it has been shown have good overall performance for any of the possible underlying modes of inheritance (additive, dominant, or recessive) (39). Potential confounders were determined a priori and included only age at baseline interview and a measure to account population stratification. Nothing else was considered to be a possible confounder because there were no other factors believed to affect both the SNPs (the exposure of interest) and adiponectin and BMI (the outcomes of interest). Ancestry allelic clusters from STRUCTURE as well as principal components from EIGENSTRAT (both derived from the set of 292 AIM SNPs) were individually examined as covariates to account for population stratification. Estimates from models using each of these approaches were found to be essentially the same and thus the STRUCTURE estimates were selected for all subsequent analyses because of the smaller degrees of freedom required. Examination of the parameter estimates from the general models was used to determine whether a different model form (additive, dominant, or recessive) was potentially more appropriate for the data. In the case that a different model form was suggested, additional models were examined using the alternate model form.

To examine interactions between the environmental factors and individual SNPs in exploratory analyses, regression models were developed that included cross-product terms for each SNP-environmental factor. These terms were assessed through likelihood ratio tests of models with and without the cross-product terms. Only the SNPs that were found to be significantly associated with adiponectin based on the single SNP-outcome analyses described above were considered for the exploratory SNP-environmental factor interaction analyses. While this study had low power to detect interactions, observed interaction will be used to generate future research projects within the SCCS and beyond.

Multiple comparisons

Multiple comparisons were an extremely important consideration in this project which included three genes each with multiple SNPs being examined in individual statistical models. In order to address the multiple testing that was conducted, a Bonferroni correction was applied. The Bonferroni correction was implemented by dividing the alpha level (set at 0.05 for these analyses) by the total number of tests that were conducted. Alpha values of 0.00096 and 0.00075 were used for white and black women, respectively, based on the final analysis of 52 and 67 SNPs.

Statistical considerations related to enrollment at Community Health Centers

Women were enrolled into the SCCS at Community Health Centers (CHCs) and thus the data can be viewed as clustered within CHCs. If there is clustering by CHC, the parameter estimates from the linear regression model may be misspecified and the variance measures may be biased downwards. Direct adjustment for CHC was deemed unfeasible due to the large number of indicator variables that would be required. An alternate method of accounting for variation resulting from the location of the CHC was devised. The location of each CHC was geocoded and then merged to the United States Census data to determine the

county location. Each county was assigned a Rural-Urban Continuum code as defined by the United States Department of Agriculture Economic Research Service (40). There are 9 Rural-Urban Continuum codes that classify metro (or urban) counties by the size of their metro area and non-metro (or rural) counties by degree of urbanization and proximity to a metro area. This 9-level variable was collapsed into a dichotomous rural/urban variable and was included in the statistical models used to examine the associations between BMI and adiponectin. There were no differences in the BMI-adiponectin effect estimates with and without adjustment for the CHC location and thus this variable was not included in the final models.

Missing data

Overall, in the SCCS there is a very small amount of missing data from baseline interview. The baseline interview data were collected using a CAPI that required each question to be answered by the study participant before the next question was asked by the study interviewer. In addition, nearly all participants (99%) completed the baseline interview. Adiponectin levels were successfully measured in 1992 out of 2,000 women included in this study (99.6%). Among the 1,992 women with measured adiponectin levels in this study, there were no missing values for BMI, age at interview, educational attainment, menopausal status, diabetes, heart attack/coronary artery disease, or hypertension. Missing values were small for household income (N=13), total physical activity (N=9), HDL-cholesterol (N=16), alcohol consumption (N=5), cigarette smoking (N=10), number of live births (N=1), age of menarche (N=12), high cholesterol (N=2), and depression (N=2). The largest source of missing data was from variables derived from the scored FFQ including total energy intake, macronutrient intake and fiber intake (N=68 missing for all). The Illumina GoldenGateTM assay was carefully selected because it has a record of a high probability of genotyping

success. Indeed, for most SNPs, all women were successfully genotyped. Only 11 SNPs were not able to be genotyped for the entire sample of women and the most common number of samples that failed genotyping was one (for 5 SNPs). Because of the small amount of missing data overall and the reasonable assumption that these data are missing completely at random, complete case analyses were conducted.

Power calculations

Statistical power was calculated for the sample of 2,000 women prior to the collection of the study data. A sample size of 1000 was assumed for all calculations because the models were stratified by race. An alpha-level of 0.05 was assumed throughout. The power calculations were generated using Proc Power in SAS/STAT software, Version 9.1 of the SAS System for Windows and the QUANTO program (41).

For specific aim 1, adiponectin as a continuous measure was the outcome. Adiponectin measures from the SCCS biomarker pilot study of 391 women were used for the power calculations. The mean adiponectin value for black women was 25.9 ug/mL (std dev=19.1) while the mean adiponectin value for white women was 16.6 ug/mL (std dev=12.4). The exposures of interest were BMI as well as environmental factors including physical activity, reproductive factors, energy and nutrient intake, alcohol consumption, smoking, and co-morbid conditions. These exposures were entered into a single multiple linear regression model with adiponectin as the outcome.

Because of interest not just in a single exposure-outcome association but also in determining which factors were predictive of adiponectin levels, the increase in the R2 value between an intercept-only model and a model with all parameters was examined. Table 2.9 shows the predicted power for various R2 values. While these R2 values are overall low, they are not inconsistent with many epidemiologic models in which predictive power is

relatively low. However, Table 2.9 shows that except for extremely small differences in R2, the study was amply powered to develop a prediction model for adiponectin.

For specific aim 2, a series of linear regression models with adiponectin as the outcome were developed. To calculate the power to detect a difference in the β coefficients for each genotype in relation to adiponectin level, a single linear regression model with adiponectin as a continuous outcome with mean 17 and standard deviation of 12.4 (based on the results from the black women in the SCCS Biospecimen Study) and a single SNP with 3 genotype categories was assumed. A Bonferroni correction was applied to the α-level of 0.05 by assuming models were be constructed for 72 typed SNPs (over the 3 genes) resulting in a final α-level of 0.05/72 = 6.9x10-4 (42). Table 2.10 shows the power calculated over a range of minor allele frequencies (MAF) (0.05 to 0.40) and β coefficients. The β coefficients represent change in adiponectin level between genotype categories; little is known about what change in adiponectin has clinical meaning making the selection of β coefficients somewhat arbitrary but for the purposes of these power calculations, values for change in adiponectin were selected that have been observed in weight loss studies and between gender and race groups (43). The MAF values were selected to represent the range of MAF values found in the selected SNPs (see tables 2.4-2.6). For changes greater than 5.0 ug/mL of adiponectin, even the lowest MAF values were well-powered. For smaller changes in adiponectin levels, only the analyses of the more common SNPs were well-powered.
For specific aim 3, a similar series of linear regression models with BMI as the outcome were developed. To calculate the power to detect a difference in the β coefficients for each genotype in relation to BMI, a single model with BMI as a continuous outcome with mean 30 and standard deviation of 6.5 (calculated from the sample of 2,000 women already selected

for the proposed research) and a single SNP with 3 genotype categories was assumed. A Bonferroni correction was applied using the final α-level of 0.05/72 = 6.9x10-4. Table 2.11 shows the power calculated over a range of minor allele frequencies (MAF) (0.05 to 0.40) and β coefficients. For a change in BMI of 2.5 or greater, there is ample power at all but the smallest MAF values. A change in BMI of 2.5 kg/m2 is equivalent to half the distance between each of the categories of BMI that have been set forth by the WHO as being correlated with negative health consequences (44). As expected, power was lowest for models in which smaller changes in β coefficients and low MAF values were assumed but the study was overall well-powered for differences in BMI that are expected to be clinically relevant (i.e. BMI changes greater than 2.5 kg/m2) for all but the rarest SNPs selected for genotyping.

Strengths and limitations

This project was uniquely poised to take advantage of the SCCS population, the only large cohort study to date that has enrolled black and white participants of a similarly low socioeconomic level. Because of the rapidly increasing obesity prevalence in the US and the large differential in the proportion of overweight and obese women by race, this work was urgently needed to further our understanding of obesity in both black and white women. Previous studies have been able to examine adiponectin in relation to obesity in white women but this was one of the first studies to examine adiponectin in relation to obesity in a large group of black and white women over a wide range of age and body size as well as to characterize associations between adiponectin and environmental/behavioral characteristics independent of obesity.

An important limitation of this study is that it was cross-sectional and thus temporality could not be assessed for the relationships examined between adiponectin,

obesity, and environmental factors. Additionally, factors measured at the time of the baseline interview (such as alcohol intake, diet, and physical activity) were assumed to reflect past exposures and to be reflective of the levels that may have influenced current body size but this cannot be determined using the cross-sectional study design.

An additional limitation of this study was that weight (and thus BMI) was not assessed over the life course for each woman. A further limitation of the BMI measurement was the use of self-reported height and weight values rather than measured values. While older literature has indicated a high concordance for measured and self-reported values of height and weight (45), a more recent review indicates that height tends to be overreported while weight tends to be underreported (46). This phenomenon may even be exaggerated in overweight women (16) leading to underestimates in BMI. However, the nature of the in-person interview and the high concordance observed between self-reported values and measured height and weight on a subset of participants in the SCCS indicates any effects of error resulting from self-reported body size were likely small. A final limitation regarding BMI is that the sampling for the proposed research excluded women with a BMI > 45 kg/m2. If the relationship between adiponectin and BMI is non-linear (i.e. the slopes are different for women in the included BM range of 18.5 – 45 kg/m2 compared to the excluded women with BMI greater than 45 kg/m2), the estimates from the models created in these analyses may not be correct for women with an extremely large body size. Thus, the results of this study are not informative as to associations with BMI at extreme levels but are useful in gaining a better understanding of these associations in the range of body size most often observed in the population.

The measurement of adiponectin was based on a single blood sample which is another limitation of the proposed design. Because of the complexity of the SCCS design, it was not feasible to obtain multiple blood samples from participants. Previous studies of adiponectin levels have frequently relied on single measurements as well. A pilot study from the Health Professionals Follow-up study found that adiponectin levels measured one year apart in a sample of 20 men were highly correlated (intraclass correlation coefficient=0.85), indicating that a single measurement of adiponectin may be sufficient for risk assessment in large epidemiologic studies (47). A study of 48 Chinese men also demonstrated that adiponectin values measured across four seasons were high correlated (intraclass correlation coefficient=0.81) (48).

Additionally, the blood samples in which adiponectin was measured were not fasting samples. Unfortunately, little research is available to determine the effects of fasting versus non-fasting blood samples on adiponectin concentration. While many small studies that have examined adiponectin in relation to insulin or glucose levels have used fasting samples (7, 10, 12), adiponectin levels have not been measured exclusively in fasting samples in large cohort studies (for example, a combination of fasting and non-fasting samples were used to measure adiponectin in the Nurses Health Study and Health Professionals Follow-up Study (49, 50)). In the current study, women were stratified by fasting status in order to address whether fasting affected the measurement of adiponectin levels. Approximately 44% of the 2,000 women in this study reported their last meal more than 8 hours before their blood draw and were considered fasting. There were no differences in the measured adiponectin levels by fasting blood status (black women fasting v. non-fasting mean adiponectin=14.9 v. 15.9, p=0.25; white fasting v. non-fasting mean=20.2 v. 19.6, p=0.6). Additionally, models were

restricted to the 421 black and 450 white women with fasting blood samples and the association between BMI and adiponectin was compared to the results for the entire sample (Table A.20 Fasting status was not a consideration for the associations examined between BMI and genetic polymorphisms in ADIPOQ, ADIPOR1, and ADIPOR2.

A further limitation of this study is related to the measurement of the covariates. Diet, alcohol, and physical activity measures were derived from instruments developed specifically for the SCCS baseline interview. Validation studies for the FFQ and the physical activity questions are currently in progress within the SCCS. Preliminary results indicate that the SCCS FFQ is able to generate useful rankings among cohort members for dietary intakes (51) and that the physical activity measures when compared to objective measures (such as accelerometer-measured activity) have reasonable validity and reliability and are comparable to other validated physical activity questionnaires (personal communication, Dr. Maciej Buchowski, 2009). Current smoking status has been validated in the SCCS Biospecimen Pilot Study using cotinine measurements in blood samples and smoking status was found to be generally well-correlated with cotinine levels (52).

Finally, the study sample used for these analyses was drawn from the larger SCCS population which enrolled participants through Community Health Centers (CHCs). Because of this method of recruitment, external validity was a potential limitation. The CHCs used for enrollment were located in the southeastern United States, and the patient population from these CHCs may differ for many characteristics from the general US population. In addition, the population that utilizes health services at CHCs is generally of lower SES and a higher proportion of patients lack health insurance than in the general population. Finally, the SCCS cohort has a higher prevalence of co-morbidities (such as diabetes and

hypertension) than the general population, limiting generalizability. Because of the inherent differences in the CHC population from the general US population, in interpreting the results of this study, care was taken so as to not to make inferences about the black or white population of the US in general. However, it should be emphasized that the use of the SCCS participants for this research was also a major strength first, because of the large number of blacks participants and second, because few previous studies have included large numbers of both black and white women of similarly low socioeconomic status and geographic location.

Summary

Adiponectin may be an important link between obesity and disease risk and may be a useful therapeutic for cardiovascular disease, cancer, and type 2 diabetes. However, limited data are available regarding adiponectin levels or its correlates in black women. Additionally, adiponectin levels are highly heritable and a number of single nucleotide polymorphisms (SNPs) in the genes encoding adiponectin (ADIPOQ) and its receptors (ADIPOR1 and ADIPOR2) have been examined in relation to circulating adiponectin levels and obesity phenotypes although again, few of these studies have included black participants. Cross-sectional interview data and blood samples collected from 2,000 women (1,000 black and 1,000 white) who enrolled in the Southern Community Cohort Study at Community Health Centers in twelve southeastern states from 2002 to 2006 were used in this project to assess adiponectin levels in relation to BMI, environmental and behavioral factors, and genetic variants in ADIPOQ, ADIPOR1, and ADIPOR2. Adiponectin levels in relation to BMI and environmental and behavioral characteristics were evaluated using race-specific linear regression models with adjustment for potential confounders. In addition 25 tag-SNPs in ADIPOQ, 19 in ADIPOR1, and 27 in ADIPOR2 were examined in relation to adiponectin levels and BMI. SNP-adiponectin and SNP-BMI associations were evaluated using race-

stratified linear regression models with adjustment for age and percentage of African ancestry to account for possible population stratification. A race-specific Bonferroni p-value threshold was employed for significance testing. This study was able to assess associations between obesity, adiponectin, and environmental and genetic correlates in the largest-to-date sample of black and white women over a range of age and body sizes from similar socioeconomic and geographic backgrounds.

Figure 2.1 Location of Community Health Centers for enrollment of participants into the Southern Community Cohort Study, 2002-2006

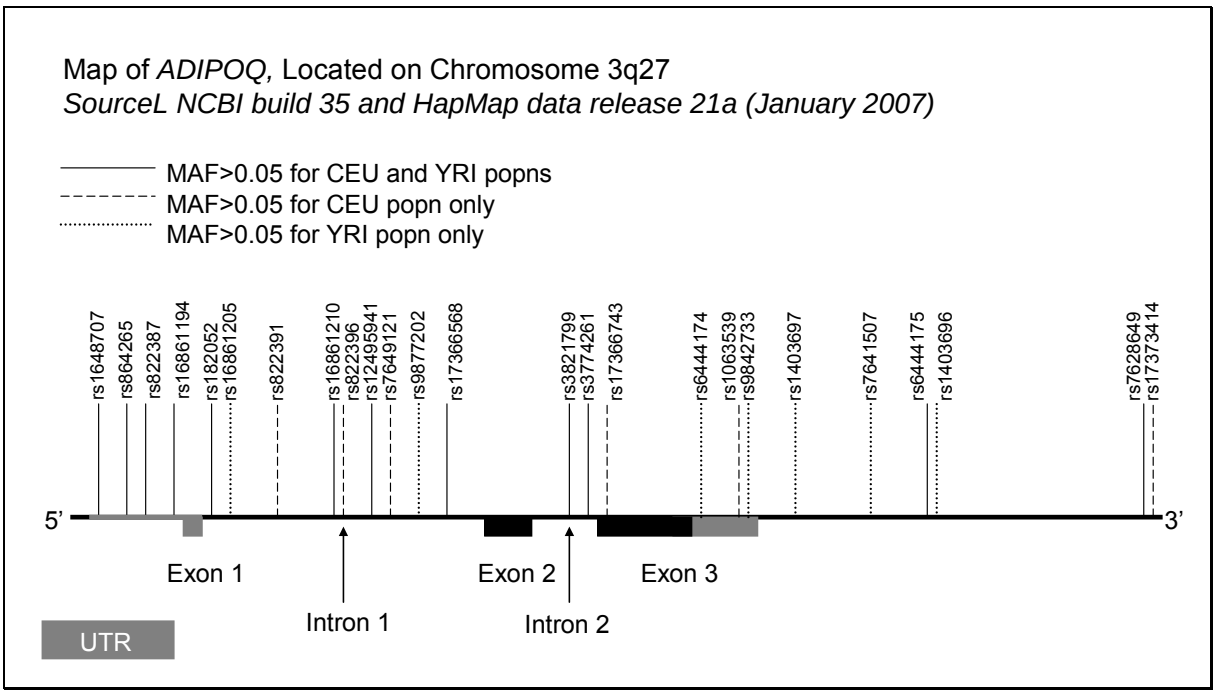

Figure 2.2 Map of SNPs selected for genotyping in *ADIPOQ*

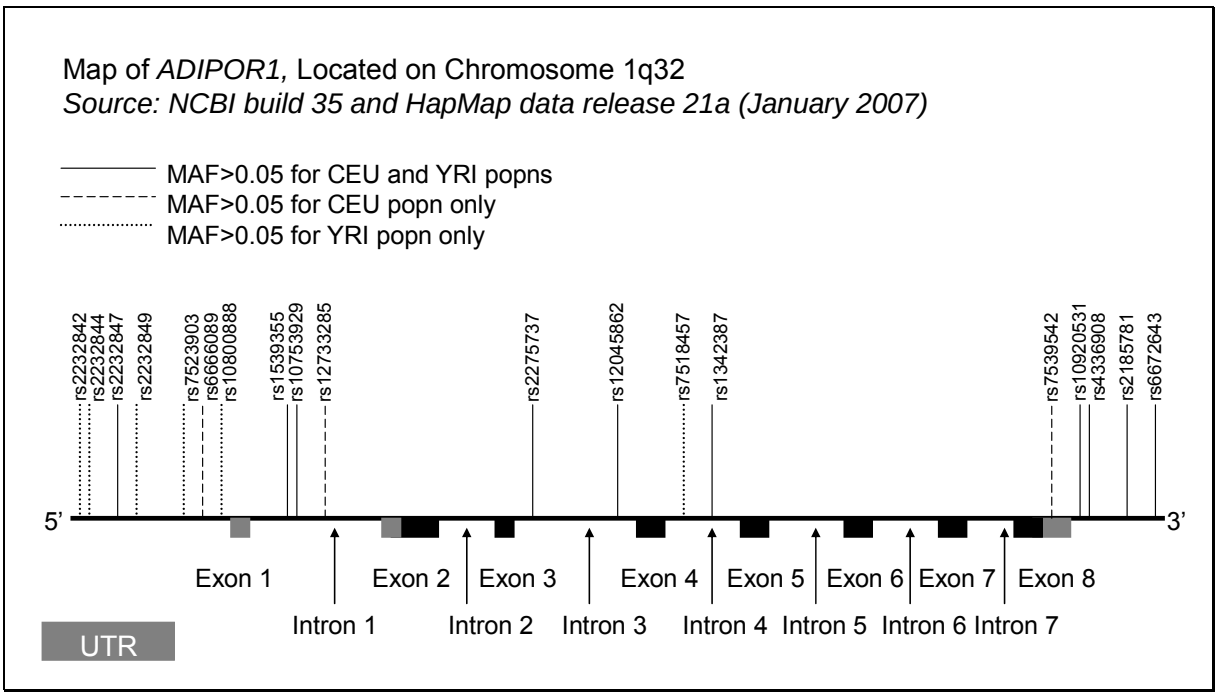

Figure 2.3 Map of SNPs selected for genotyping in *ADIPOR1*

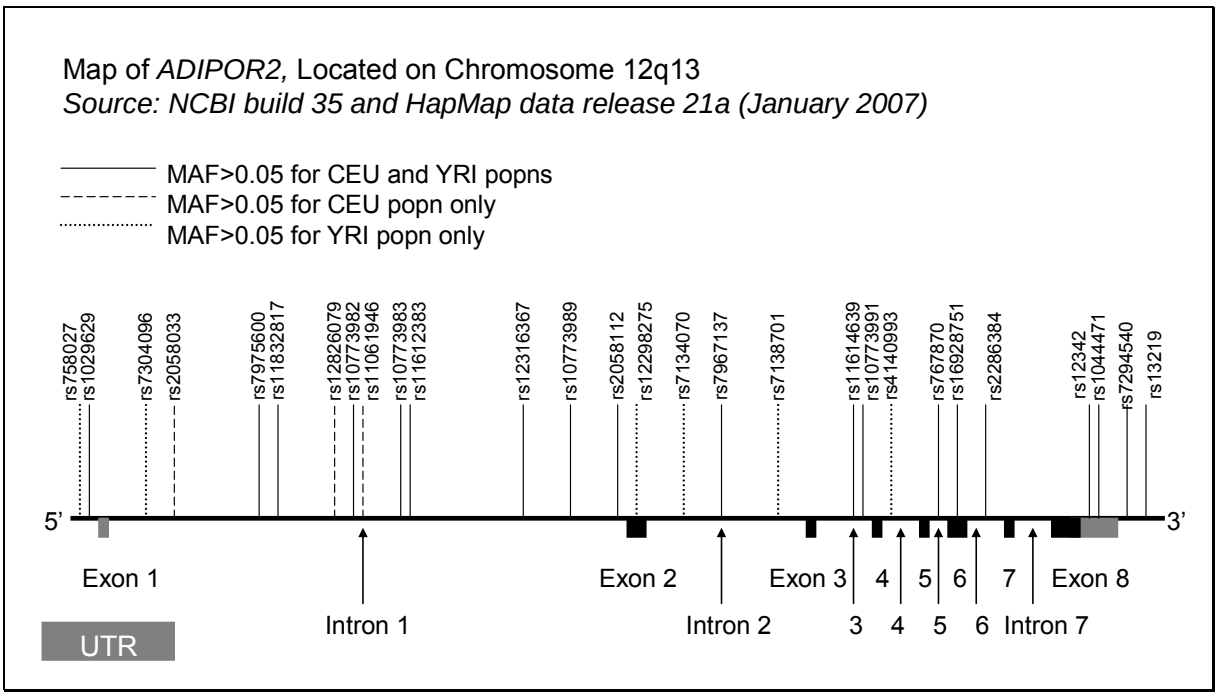

Figure 2.4 Map of SNPs selected for genotyping in *ADIPOR2*

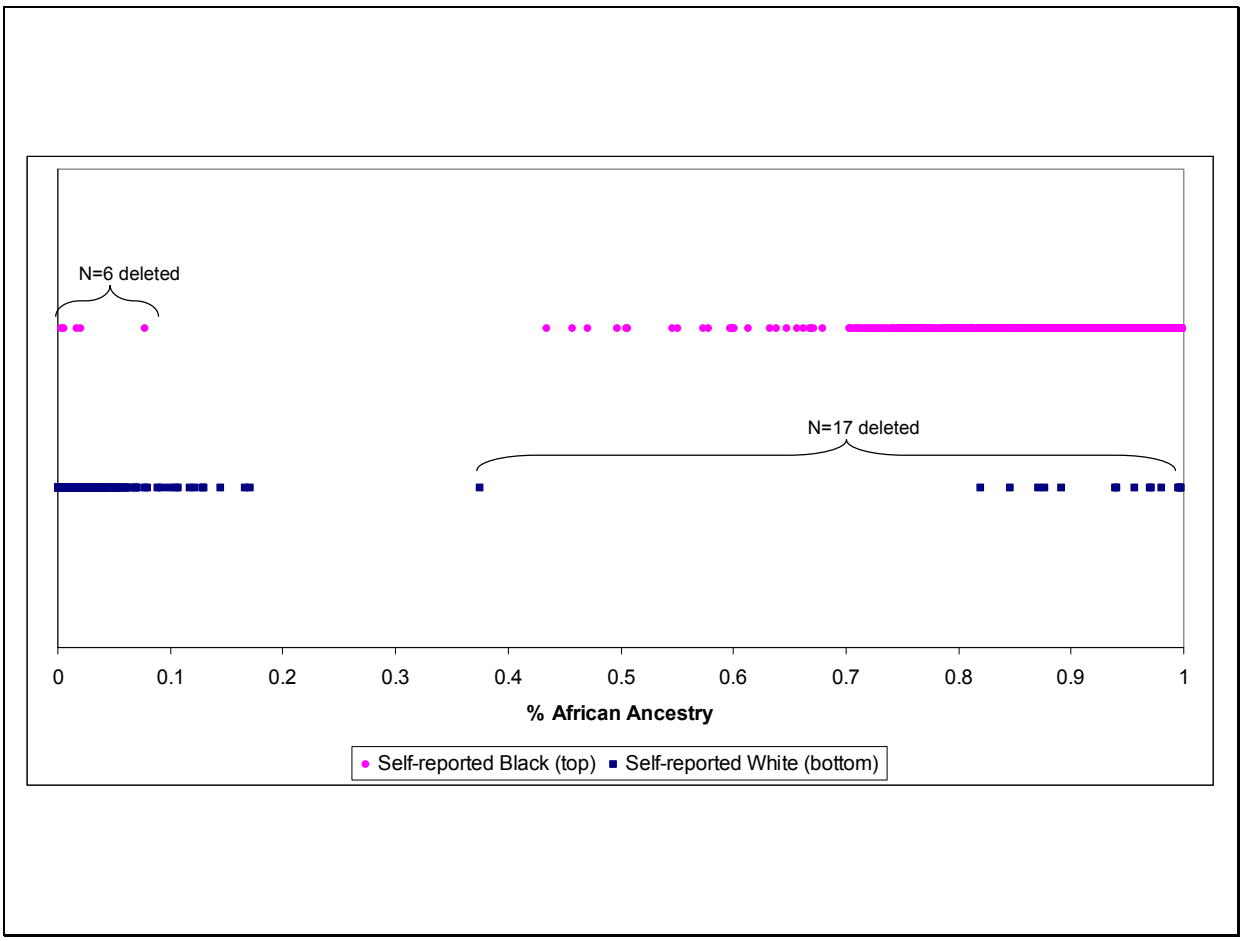

Figure 2.5 Percentage of African ancestry as estimated by STRUCTURE from 292 Ancestry Informative Markers for black women (N=996) and white women (N=994) successfully genotyped for *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* by self-reported race categories (black and white).

Table 2.1 Selected characteristics for female participants enrolled in the Southern Community Cohort Study through February 2006

	Black women N=20,635		White women N=6,574	
	N	**%**	**N**	**%**
Age at interview				
40 – 49	10,271	49.8	2,743	41.7
50 – 59	6,612	32.0	2,214	33.7
60 – 69	2,777	13.5	1,232	18.7
70 – 79	975	4.7	385	5.9
Household income				
< $15K	12,627	62.0	3,853	59.3
$15 - $25K	4,863	23.9	1,294	19.9
$25 - $50K	2,223	10.9	816	12.6
> $50K	658	3.2	531	8.2
Education (yrs)				
< 9	1,628	7.9	612	9.3
9-11	5,001	24.2	1,368	20.8
12	8,143	39.5	2,643	40.2
> 12	5,855	28.4	1,948	29.7
Health Insurance				
None	2,744	42.1	7,935	38.7
Private	1,540	23.6	4,726	23.1
Medicare/Medicaid	1,519	23.3	5,698	27.8
Other1	714	11.0	2,124	10.4
Diabetes2				
Yes	5,044	24.5	1,342	20.5
No	15,556	75.5	5,214	79.5
Hypertension3				
Yes	12,989	63.0	3,365	51.3
No	7,613	37.0	3,189	48.6
BMI (kg/m2)				
< 18.5	217	1.1	131	2.0
18.5 – 24.9	3,299	16.2	1,535	23.5
25.0 – 29.9	5,202	25.5	1,714	26.3
30.0 – 34.9	5,101	25.0	1,424	21.8
35.0 – 39.9	3,367	16.5	861	13.2
> 40.0	3,215	15.8	860	13.2

[1] Reported as Military/VA or 'other' insurance

[2] Has a doctor ever told you that you have had diabetes or high blood sugar? (not during pregnancy)

[3] Has a doctor ever told you that you have had high blood pressure?

Table 2.2 Characteristics of 28,158 female Southern Community Cohort Study (SCCS) participants enrolled through February 2006 who did and did not provide blood samples at the baseline SCCS interview

	Provided blood sample N=14,093		**No blood sample provided N=14,065**	
	N	**%**	**N**	**%**
Race				
Black	9,961	70.7	10,674	75.9
White	3,655	25.9	2,919	20.8
Other	459	3.3	378	2.7
Missing	18	0.1	94	0.7
Age at interview				
40 – 49	6,827	48.4	6,606	47.0
50 – 59	4,556	32.3	4,606	32.8
60 – 69	2,110	15.0	2,046	14.6
70 – 79	600	4.3	807	5.7
Missing	0	0.0	0	0.0
Household income				
< $15K	8,623	61.2	8,341	59.3
$15 - $25K	3,219	22.8	3,105	22.1
$25 - $50K	1,582	11.2	1,579	11.2
> $50K	501	3.6	748	5.3
Missing	168	1.2	292	2.1
Education (yrs)				
< 9	1,178	8.4	1,145	8.1
9-11	3,314	23.5	3,182	22.6
12	5,635	40.0	5,428	38.6
> 12	3,945	28.0	4,216	30.0
Missing	21	0.2	94	0.7
BMI (kg/m^2)				
< 18.5	135	1.0	227	1.6
18.5 – 24.9	2,390	17.0	2,618	18.6
25.0 – 29.9	3,515	24.9	3,625	25.8
30.0 – 34.9	3,436	24.4	3,289	23.4
35.0 – 39.9	2,262	16.1	2,073	14.7
$\geq$ 40.0	2,210	15.7	1,985	14.1
Missing	145	1.0	248	1.8

Note: Women who reported hepatitis or HIV infection are not included in this table because they were not eligible to provide a blood sample due to shipping restrictions.

Table 2.3 Descriptive statistics for adiponectin levels (ug/ml) measured in the Southern Community Cohort Study biospecimen pilot study among females

	Black women	**White women**
Mean	16.6	25.9
Median	13.0	19.3
Std. deviation	12.4	19.1
Range	1.9 – 86.7	0.0 – 98.0

Table 2.4 Characteristics of female Southern Community Cohort Study (SCCS) participants enrolled in the SCCS through February 2006 who provided a blood sample (N=14,093), were eligible for selection into the proposed research after exclusionary criteria were applied (N=10,585), and were selected for the final sample (N=2,000)

	All women with blood N=14,093		**Eligible for sample selection N=10,585**		**Selected for proposed research N=2,000**	
	N	**%**	**N**	**%**	**N**	**%**
Race						
Black	9,961	70.7	7,805	73.7	1,000	50.0
White	3,655	25.9	2,780	26.3	1,000	50.0
Other	459	3.3	0	0.0	0	0.0
Missing	18	0.1	0	0.0	0	0.0
Age at interview						
40 – 49	6,827	48.4	5,078	48.0	1,183	59.2
50 – 59	4,556	32.3	3,410	32.2	511	25.6
60 – 69	2,110	15.0	1,650	15.6	266	11.3
70 – 79	600	4.3	447	42	80	4.0
Household income						
< $15K	8,623	61.2	6,454	61.0	1,219	61.0
$15 - $25K	3,219	22.8	2,477	23.4	441	22.1
$25 - $50K	1,582	11.2	1,186	11.2	246	12.3
> $50K	501	3.6	360	3.4	81	4.0
Missing	168	1.2	108	1.0	13	0.7
Education (yrs)						
< 9	1,178	8.4	873	8.3	164	8.2
9-11	3,314	23.5	2,507	23.7	467	23.4
12	5,635	40.0	4,284	40.5	827	41.4
> 12	3,945	28.0	2,918	27.6	542	27.1

Table 2.5. Descriptive statistics for HDL-cholesterol values by study sample and race

	HDL	
	White	**Black**
Total		
N	988	988
Mean (Std dev)	49.5 (13.1)	55.9 (15.4)
Median (Q1-Q3)	48 (41-55)	53 (45-64)
Pilot Sample		
N	195	195
Mean (Std dev)	52.0 (13.6)	57.9 (4835)
Median (Q1-Q3)	50 (42-59)	55 (47-67)
Komen Sample		
N	793	793
Mean (Std dev)	48.9 (12.9)	55.4 (15.4)
Median (Q1-Q3)	47 (40-55)	53 (45-63)
Fasting		
N	445	417
Mean (Std dev)	49.1 (13.9)	55.5 (14.8)
Median (Q1-Q3)	48 (40-55)	53 (46-62)
Non-Fasting		
N	543	570
Mean (Std dev)	49.8 (12.4)	56.2 (15.9)
Median (Q1-Q3)	48 (41-57)	54 (45-64)
p-value[1]	*0.42*	*0.51*

[1] p-value from t-test comparing fasting mean to non-fasting mean within each stratum of race

Table 2.6 Single nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene selected for genotyping

SNP	Caucasian MAF[1,4]	African MAF[2,4]	African-American MAF[3,4]
rs1648707	0.37	0.36	
rs864265	0.13	0.15	
rs822387	0.10	0.35	
rs16861194	0.07	0.31	
rs182052	0.35	0.40	0.45
rs16861205	0.04	0.22	
rs822391	0.17	0.00	
rs16861210	0.10	0.16	
rs822396	0.15	0.00	
rs12495941	0.31	0.39	
rs7649121	0.25	0.00	
rs9877202	0.01	0.20	
rs17366568	0.15	0.05	
rs3821799	0.46	0.39	
rs3774261	0.38	0.43	
rs17366743	0.08	0.00	
rs6444174	0.00	0.24	
rs1063539	0.13	0.01	
rs9842733	0.00	0.14	
rs1403697	0.00	0.20	
rs7641507	0.00	0.06	
rs6444175	0.29	0.28	
rs1403696	0.00	0.30	
rs7628649	0.10	0.38	
rs17373414	0.11	0.00	

[1] From HapMap CEU population

[2] From HapMap YRI population

[3] From TSC 42 African-American population

[4] MAF = Minor allele frequency

Table 2.7 Single nucleotide polymorphisms (SNPs) in the *ADIPOR1* gene selected for genotyping

SNP	Caucasian MAF[1,3]	African MAF[2,3]
rs6672643	0.12	0.33
rs2185781	0.21	0.31
rs4336908	0.19	0.10
rs10920531	0.33	0.29
rs7539542	0.29	0.00
rs1342387	0.45	0.49
rs7518457	0.00	0.09
rs12045862	0.23	0.08
rs2275737	0.44	0.48
rs12733285	0.26	0.00
rs10753929	0.20	0.23
rs1539355	0.33	0.42
rs10800888	0.00	0.17
rs6666089	0.33	0.00
rs7523903	0.00	0.30
rs2232849	0.00	0.13
rs2232847	0.33	0.13
rs2232844	0.00	0.06
rs2232842	0.01	0.15

[1] From HapMap CEU population

[2] From HapMap YRI population

[3] MAF = Minor allele frequency

Table 2.8 Single nucleotide polymorphisms (SNPs) in the *ADIPOR2* gene selected for genotyping

SNP	Caucasian MAF[1,3]	African MAF[2,3]
rs758027	0.00	0.13
rs1029629	0.32	0.23
rs7304096	0.00	0.06
rs2058033	0.15	0.00
rs7975600	0.13	0.14
rs11832817	0.29	0.15
rs12826079	0.09	0.00
rs10773982	0.33	0.37
rs11061946	0.07	0.00
rs10773983	0.35	0.13
rs11612383	0.32	0.27
rs12316367	0.49	0.10
rs10773989	0.47	0.30
rs2058112	0.13	0.13
rs12298275	0.00	0.08
rs7134070	0.01	0.14
rs7967137	0.13	0.24
rs7138701	0.01	0.21
rs11614639	0.46	0.31
rs10773991	0.48	0.23
rs4140993	0.01	0.20
rs16928751	0.13	0.13
rs2286384	0.48	0.39
rs12342	0.33	0.14
rs1044471	0.47	0.22
rs7294540	0.46	0.08
rs13219	0.44	0.18
rs2058111	0.42	0.24

[1] From HapMap CEU population

[2] From HapMap YRI population

[3] MAF = Minor allele frequency

Table 2.9 Power for Specific aim 1

R^2 value between full model and intercept-only model	Predicted Power
0.20	>0.99
0.10	>0.99
0.05	0.99
0.04	0.98

Table 2.10 Power for Specific Aim 2 (adiponectin as continuous outcome)

	Minor Allele Frequency				
β coefficient	**0.05**	**0.10**	**0.20**	**0.30**	**0.40**
2.5	0.05	0.19	0.52	0.72	0.81
5.0	0.64	0.97	>0.99	>0.99	>0.99
7.5	0.99	>0.99	>0.99	>0.99	>0.99

Table 2.11 Power for Specific Aim 3 (BMI as continuous outcome)

	Minor Allele Frequency				
β coefficient	**0.05**	**0.10**	**0.20**	**0.30**	**0.40**
1.50	0.09	0.32	0.72	0.88	0.93
2.50	0.57	0.95	>0.99	>0.99	>0.99
5.00	>0.99	>0.99	>0.99	>0.99	>0.99

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CHAPTER 3: SERUM ADIPONECTIN IN RELATION TO BODY MASS INDEX AND OTHER PREDICTORS IN BLACK AND WHITE WOMEN

Abstract

Adiponectin is a promising biomarker linking obesity and disease risk and may be a useful therapeutic for cardiovascular disease, cancer, and type 2 diabetes. However, limited data are available regarding adiponectin in black women among whom obesity is highly prevalent. We conducted a cross-sectional analysis in Southern Community Cohort Study participants to assess racial differences and correlates of serum adiponectin. Adiponectin was measured in 996 black and 996 white women age 40-79 years recruited at community health centers across the southeastern US from 2002 to 2006. Blacks had significantly lower adiponectin levels than whites (geometric means 11.7 versus 15.1 ug/ml, $p<0.0001$). Among blacks, adiponectin levels were lower among overweight and obese women compared to healthy weight women but showed no clear decreasing trend with increasing severity of obesity; adjusted geometric means (95% confidence interval) were 15.0 (13.8-16.4), 11.5 (10.6-12.5), 9.7 (9.0-10.6), 11.4 (10.3-12.6), and 10.9 (9.5-12.6) ug/ml for body mass index [BMI] categories of 18.5-24.9, 25-29.9, 30-34.9, 35-39.9, and 40-45. In contrast, among whites there was a monotonic reduction in adiponectin across all BMI categories (adjusted geometric means = 19.9 (18.3-21.7), 15.1 (13.9-16.4), 14.3 (13.2-15.5), 12.5 (11.2-13.9), and 11.0 (9.7-12.5) ug/ml). In multiple linear regression models, BMI, age, HDL-cholesterol, and hypertension were important predictors of adiponectin in both groups whereas socioeconomic status (education and income), cigarette smoking, physical activity, and

dietary, reproductive, and co-morbidity indices were not significant predictors in either black or white women. Our results indicate that racial differences exist in both the magnitude and form of the adiponectin-BMI association.

Introduction

Adiponectin is a protein produced exclusively in adipose tissue that appears to play a critical role in mediating physiological effects such as insulin sensitivity, inflammatory response, and cell proliferation. Adiponectin levels are inversely associated with obesity and are thought to decrease in individuals with increased adiposity through down-regulation of adiponectin receptors (1). Adiponectin may also be affected by diet, physical activity, comorbidities, and other environmental factors as well as variation in genes encoding adiponectin or its receptors (1). Because adiponectin is inversely associated with obesity phenotypes as well as several obesity-related diseases (2), there is speculation that adiponectin activity may be a useful target in the prevention of cardiovascular disease, cancer, and type 2 diabetes (1, 2). With the highest prevalence of obesity found among non-Hispanic black women (39%) and the lowest among non-Hispanic white women (22%) (3), differences in adiponectin levels across race groups may contribute to disparities in obesity-related diseases between black and white women.

Identifying correlates of adiponectin and ascertaining whether they vary by race is likely to enhance the development of effective adiponectin-related chemopreventive strategies. Thus, the goal of this analysis was to examine associations between adiponectin levels, body mass index (BMI), and other potential correlates and to assess whether associations varied by race.

Methods and Procedures

Institutional Review Boards at Vanderbilt University, Meharry Medical College, and the University of North Carolina at Chapel Hill approved this study.

Study population

The Southern Community Cohort Study (SCCS) is a prospective epidemiologic cohort study designed to examine racial disparities in cancer incidence and mortality (4). Study enrollment began in 2002 in 12 southeastern states at Community Health Centers (CHC) which are government-funded facilities providing health services primarily to low-income individuals in medically underserved areas (5). As described previously (4), participants were required to be age 40-79 years, English-speaking, and not have undergone treatment for cancer within the past year. From over 47,000 participants enrolled through early 2006, a sub-sample of 2,000 women who provided a blood sample at study enrollment and self-reported their race as either 'Black/African American' or 'White' was selected for further biomarker analyses. This included a random sample of 395 women selected in 2005 within three strata (race, BMI, and smoking status) and a second random sample of 1,605 women selected in 2006 in equal numbers across race, BMI, and menopausal status categories.

Data collection

Trained study interviewers led all participants through a structured questionnaire using a computer-assisted interview with extensive skip patterns and range and logic checks. The interview elicited information including demographics, anthropometrics, and several aspects of health and behavior. Physical activity was measured using a questionnaire developed for the SCCS to comprehensively assess active and sedentary behaviors at the time of the interview. Dietary intake in the year prior to the baseline interview was measured using an 89-item food frequency questionnaire (FFQ) designed specifically for the SCCS to

elicit information about foods most commonly eaten in the southeastern United States (6, 7). For the 20% of women who were patients in the CHC on the day of the baseline interview, measured height and weight were abstracted from medical records for validation purposes.

A convenience blood sample was collected at the time of recruitment using one EDTA-containing plasma tube and one serum BD Vacutainer® tube. For this study, the median time between the last reported meal and blood collection was 6.0 for blacks and 6.3 for whites (p=0.07). Fasting blood, defined as at least 8 hours since last meal, was collected for 44% of the participants. Blood samples were shipped cold to Vanderbilt University in Nashville, TN, where they were processed for storage at -80° C. 84% of the blood samples were received the day after the blood draw and 98% were received within two days. The samples were frozen for an average of 2.6 years (range 3 months to 5 years) prior to analysis.

Laboratory assays

Adiponectin levels were measured in serum by immunoassay using the LINCOplex kit (Luminex® xMAP™ Technology, St. Louis, MO) in the Vanderbilt Hormone Assay and Analytical Services Core Laboratory in duplicate for each woman. The average of the two measurements was used in all analyses. Duplicate sets of samples for five randomly selected women as well as five repeat samples from each of two pooled samples were measured to assess the reliability and validity of the assay. Adiponectin levels were successfully measured in 1,992 of the 2,000 samples (eight samples failed due to a filter plate error or low sample volume). The intra-assay coefficient of variation was 9.4%. High-density lipoprotein (HDL) cholesterol was measured in serum by the Vanderbilt Lipid Laboratory using the ACE Clinical Chemistry System and the ACE HDLC Reagent (#SA1038) following the manufacturer's protocols (Alfa Wassermann, Inc, West Caldwell, NJ). The intra-assay coefficient of variation was 1.6%. Neither adiponectin nor HDL-cholesterol was associated

with fasting status (for adiponectin, p=0.3 for blacks, p=0.6 for whites; for HDL, p=0.5 for blacks, p=0.4 for whites).

Statistical Methods

For this cross-sectional analysis, data from 1,992 women with measured adiponectin were analyzed. BMI was calculated from self-reported values as [weight (kg)] / [height $(m)^2$]. Dietary intakes, total physical activity, and HDL-cholesterol were categorized into quartiles based on the distribution of the entire sample. Other characteristics were categorized as shown in Table 3.1. The Wilcoxon signed rank test was used to compare adiponectin levels between groups.

Adiponectin had a skewed distribution (Figure 3.1), and therefore was log-transformed to better meet modeling assumptions (8). To ease presentation, back-transformed values are shown. Linear regression models were constructed for blacks and whites separately. All models were adjusted for sample selection (395 selected in 2005 versus 1605 selected later) and age at baseline interview. Further adjustment for factors used in the sample selection (cigarette smoking status and menopausal status) did not alter the results and were not included in the models presented here. Categorized covariates with inherent order were modeled using indicator variables rather than as ordinal variables because the assumption of linearity was not generally met.

The first analytic objective was to characterize the relationship between adiponectin and BMI within each race group. Continuous BMI was regressed on adiponectin, and potential confounders (determined from the literature and categorized as shown in Table 3.1) were added to the model using backwards model selection with a change-in-estimate criterion of $\geq$ 5%. Potential modifiers of the BMI-adiponectin relationship were assessed using the likelihood ratio test (LRT) to compare models with and without interaction terms

for BMI and potential modifiers. Adjusted geometric means for adiponectin were calculated from linear regression models that included standard categories of BMI (<18.5, 18.5-24.9, 25-29.9, 30-34.9, 35-39.9, and 40-45 kg/m^2) and the final set of confounders.

The second analytic goal was to determine predictors of adiponectin after adjustment for BMI. BMI was forced into the linear regression model and potential predictors were then added sequentially. Prediction models were compared using Akaike's Information Criterion (AIC) which balances model fit with model complexity (9), and potential predictors were included in the final model as long as their addition resulted in an AIC value at least one unit lower than the AIC for the smaller-order model.

SAS/STAT software, Version 9.1 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

Results

Adiponectin levels were nearly 25% lower among black women compared to white women (geometric means: 11.7 versus 15.1 ug/ml, respectively, $p<0.0001$). After adjustment for BMI and age, adiponectin was still significantly lower in blacks ($p<0.0001$). Unadjusted adiponectin levels decreased with increasing BMI categories in both groups although not as consistently among blacks (Table 3.1). There was a strong positive association between adiponectin and both age and HDL-cholesterol while adiponectin was slightly lower among women reporting diabetes or hypertension. Unadjusted adiponectin levels increased with increasing alcohol consumption in whites but not in blacks. Adiponectin was not consistently associated with education, income, physical activity, cigarette smoking, or any of the dietary or reproductive indices.

Figure 3.2 illustrates race-specific associations between BMI and adiponectin adjusted for HDL-cholesterol, the only variable found to be a confounder of the BMI-

adiponectin relationship. Adjusted mean adiponectin levels for blacks were lower than those for whites within each category of BMI although the differences for women in the highest categories of BMI (35-39.9 and 40+) were small. Among blacks, adiponectin decreased over BMI values up to 30-34.9 before leveling off at the highest levels of BMI. In contrast, among whites, adiponectin declined steadily over increasing categories of BMI. Menopausal status did not modify the BMI-adiponectin relationship (LRT p=0.9 for blacks, p=0.6 for whites) (data not shown). In models restricted to 420 black and 450 white women with fasting blood samples, the association between BMI and adiponectin was unchanged for both groups.

Table 3.2 shows race-specific prediction models for adiponectin. Based on the AIC values, BMI was an important predictor of adiponectin in both race groups although the regression coefficient was larger in magnitude among whites. Age, HDL-cholesterol, and hypertension were also predictive of adiponectin in both groups. None of the other factors examined added additional predictive value.

Discussion

In this largest to-date, cross-sectional study of black and white women from similar geographic and socioeconomic situations, we observed that blacks had lower adiponectin levels than whites even after adjustment for BMI. Our results expand upon previous studies that have also found lower levels of adiponectin in blacks but were limited either by small numbers of black participants (10-13) or limited age ranges (14-16), indicating that racial differences in adiponectin exist across the adult age spectrum.

Adiponectin has consistently been found to be negatively correlated with obesity in white and Asian populations (17-19). Despite racial differences in the prevalence of obesity and risk for obesity-related disease, few large studies have examined adiponectin in relation

to obesity in blacks. As did our study, several small studies (11, 13, 20) as well as the Atherosclerosis Risk in Communities Study (14) found that adiponectin decreased over categories of BMI in blacks and that adjusted adiponectin values were lower for black women compared to white women in each BMI category. Our analysis included larger numbers of women with higher values of BMI than in previous studies, and we showed for the first time that the form of the BMI-adiponectin association may differ by race with white women showing a consistent decline in adiponectin over all levels of BMI while among blacks, adiponectin was lower among overweight and obese women compared to healthy weight women but there was little trend with increasing severity of obesity.

Our first-ever extensive examination of potential predictors of adiponectin found few factors that were strongly predictive of adiponectin. Adiponectin levels rose with advancing age, consistent with earlier studies (17, 21). The direction of this association has previously been noted to be paradoxical; abdominal fat, which is inversely associated with adiponectin, increases with age indicating that adiponectin levels should decrease with age. One potential explanation for this seeming contradiction is that estrogen, thought to inhibit adiponectin, decreases with age allowing adiponectin to rise (17). We also observed a strong positive association between HDL-cholesterol and adiponectin, as have others (17, 21). Mechanistically, it is hypothesized that decreased adiponectin levels may affect hepatic insulin resistance leading to increased hepatic lipase activity and decreased HDL levels (17, 21). While the cross-sectional nature of our data did not allow us to examine the temporality of the HDL-adiponectin relationship, our data demonstrate that this strong association holds across race and age groups.

Low adiponectin levels have been inversely linked to hypertension (22-24) including in one study of blacks (24), a finding we also observed in both race groups. A few prior studies have found this association only among participants with insulin resistance, but at least some evidence indicates that low adiponectin levels may affect the development of hypertension at an early stage, without involvement of insulin resistance (23). We found that adiponectin levels were only slightly lower among diabetics, and after adjustment for BMI and age, diabetes and adiponectin were not significant associated. This was somewhat unexpected since adiponectin and diabetes incidence have previously been shown to be inversely linked (25). One explanation may be that many SCCS women with prevalent diabetes were receiving treatment for their diabetes, thus reducing effects of hyperinsulinemia on adiponectin levels.

Neither physical activity nor dietary factors were predictive of adiponectin levels despite their known roles as major components of energy balance. In contrast, a prior study found that physical activity was associated with increased adiponectin levels, with moderate/high intensity activity showing stronger effects than low intensity activity (26). It is possible that the activity levels in our population were too low overall to detect effects on adiponectin levels; in fact, less than 20% of the women in either race group in this study met the recommended guidelines for physical activity (27). Little research has been conducted regarding associations between dietary factors and adiponectin. One study found no association between total calories or macronutrient intake and adiponectin (28) while fiber was found to be positively associated with adiponectin among diabetics (29, 30). It seems likely that there are many intermediaries in the pathways linking diet and physical activity to adiponectin, making the detection of associations difficult in our cross-sectional dataset.

Additionally, it is possible that once age, BMI, and HDL-cholesterol were included in models for adiponectin, minimal additional predictive value was added by factors such as diabetes, diet, and physical activity which are reasonably expected to be predictive of adiponectin but are also strongly associated with age, BMI, and HDL-cholesterol. While standard in large epidemiologic studies, the physical activity and dietary intakes obtained via questionnaire are known to contain substantial measurement error which may have resulted in the attenuation of observed associations with adiponectin.

Inferences from this study should also be considered in light of potential limitations related to our measurement of adiponectin. The high-molecular weight (HMW) form of adiponectin has been suggested to be the more biologically active (31) and thus, we may have been unable to detect certain associations because we (like most other large, population-based studies) did not specifically measure HMW adiponectin. Additionally, adiponectin was measured only at a single point in time. Studies in white and Chinese individuals found that adiponectin levels measured one year apart were highly correlated, indicating that a single measurement of adiponectin is likely sufficient for large epidemiologic studies (32, 33). Another possible limitation of our adiponectin measurement is that it was not conducted exclusively in fasting samples; however, analyses limited to samples provided more than 8 hours since the last meal did not show any appreciable differences from those using the entire population.

The use of self-reported height and weight measures is also a potential limitation. A recent review indicates that among women, height tends to be over-reported and weight under-reported (34). However, data from the 1999-2004 National Health and Examination Survey show that despite errors in self-report, BMI categories based on self-reported values

demonstrate good agreement with BMI categories from measured values (35). These data also showed that under-reporting was more common in whites and among well-educated women (35) which suggests that the BMI values calculated from self-report in the SCCS may be less vulnerable to bias than in other studies of more educated, white participants. Furthermore, in the SCCS, BMI values calculated from self-reported height and weight were very highly correlated with BMI values calculated from medical record data overall (Pearson correlation coefficient > 0.95) as well as across strata of race and BMI, indicating that our self-reported values provide a useful measure of BMI in the context of an epidemiologic study.

A major strength of this study is the utilization of the SCCS resource. By design, the black and white participants arose from similar geographic and SES backgrounds facilitating the examination of racial differences by minimizing the potential role of SES-driven confounding, a limitation that clouds the interpretation of results from many previous studies. Our study also overcomes limitations from previous studies which were hampered by small numbers of black participants, narrow age ranges, and few participants with BMI greater than 35.

Analysis of this large population of highly comparable black and white women shows that adiponectin levels are lower in blacks than in whites and that adiponectin is inversely associated with obesity but the shape of the BMI-adiponectin association differs by race. Additionally, we demonstrated that age, HDL-cholesterol, and hypertension are strong correlates of adiponectin in both race groups. Future work within the SCCS and other studies with diverse populations will be guided by these findings as the mechanistic role played by adiponectin in the development of disease is examined. Further, efforts to develop

interventions that can alter adiponectin levels for the prevention of diseases such as cardiovascular disease, cancer, and diabetes may be guided by the racial differences in adiponectin levels and its correlates observed in this study.

Acknowledgements

This project was funded in part by grant OP05-0927-DR1 from Susan G. Komen for the Cure. The Southern Community Cohort Study is funded by grant R01 CA92447 from the National Cancer Institute (NCI). Ms. Cohen also received support from NCI Training Grants T32 CA09330-26 and 5-R25-CA057726. Dr. Gammon is supported in part from P30ES10126 from the National Institute of Environmental Health Sciences. Serum sample preparation was conducted at the Survey and Biospecimen Shared Resource that is supported in part by the Vanderbilt-Ingram Cancer Center (P30 CA68485).

Table 3.1. Descriptive statistics for unadjusted serum adiponectin levels (ug/ml) characteristics among 1,992 women in the Southern Community Cohort Study, 2002-2006

	Black women				White women			
	N	Geometric Mean	25th-75th percentile		N	Geometric Mean	25th-75th percentile	
All participants	996	11.7	7.0	19.1	996	15.1	9.0	24.7
Body mass index (kg/m^2)								
< 18.5	8	19.2	8.8	36.2	10	27.1	13.5	48.3
18.5 – 24.9	240	16.1	9.6	25.7	239	22.2	13.6	36.6
25.0 – 29.9	249	11.9	7.4	18.7	248	15.6	9.8	25.0
30.0 – 34.9	250	9.5	5.9	14.4	249	13.5	8.7	20.9
35.0 – 39.9	165	10.7	6.0	17.9	146	11.9	7.7	18.5
40.0 +	85	9.8	6.0	16.6	104	9.9	5.9	15.4
Age at interview								
40 – 44	330	10.8	6.5	18.0	341	13.3	8.5	20.8
45 – 49	265	11.4	6.7	18.7	243	14.0	8.2	21.6
50 – 54	148	12.1	7.2	19.7	136	16.1	8.6	28.0
55 – 59	105	12.4	7.3	21.5	120	16.7	10.1	30.1
60 – 64	53	13.3	8.6	19.1	93	17.6	10.6	28.2
65 – 69	50	13.9	7.4	25.0	29	19.4	11.8	32.9
70 – 74	25	13.9	8.4	25.3	24	23.8	13.7	43.3
75 – 79	20	16.1	10.4	21.4	10	32.2	24.8	51.4
Household income								
< $15K	620	11.8	7.0	19.0	594	14.9	9.4	24.1
$15 - $25K	236	11.5	6.9	18.3	205	14.7	8.6	25.3
$25 - $50K	115	12.8	6.8	20.6	129	16.4	8.6	29.9
> $50K	16	10.5	6.1	18.8	64	15.8	11.0	22.9

Table 3.1 (continued). Descriptive statistics for unadjusted serum adiponectin levels (ug/ml) characteristics among 1,992 women in the Southern Community Cohort Study, 2002-2006

	Black women				White women			
	N	Geometric Mean	25th-75th percentile		N	Geometric Mean	25th-75th percentile	
Education (years)								
< 9	78	13.2	7.7	22.4	85	14.5	8.3	22.0
9-11	241	11.8	7.1	18.9	224	14.5	8.9	25.2
12	415	12.0	7.2	19.8	411	15.4	9.0	25.5
> 12	262	10.8	6.1	18.2	276	15.3	9.4	22.7
Physical activity (Met-hrs/day)								
Q1 (<10.2)	248	12.5	7.5	20.3	247	14.3	8.7	21.9
Q2 (10.2-18.3)	256	11.8	6.9	19.5	240	16.3	9.8	27.2
Q3 (18.4-29.2)	255	11.4	6.9	18.5	242	14.9	8.8	24.9
Q4 (>29.2)	233	11.4	6.8	18.5	262	15.2	8.8	25.7
HDL cholesterol (mg/dl)								
Q1 (<43)	170	7.6	5.0	10.8	314	10.4	6.6	16.8
Q2 (43-50)	231	9.4	5.8	14.9	272	14.6	9.6	21.9
Q3 (51-60)	245	13.7	8.5	21.4	220	17.6	10.8	29.4
Q4 (>60)	342	15.1	9.3	24.4	182	24.8	16.2	40.9
Total energy intake (kcal/day)								
Q1 (<1352)	213	10.9	6.2	18.0	268	16.0	9.2	27.2
Q2 (1352-1875)	226	12.4	7.6	18.9	254	15.4	8.8	26.5
Q3 (1876-2644)	217	12.5	7.2	22.7	264	14.4	8.9	21.9
Q4 (>2644)	294	11.5	7.2	18.0	187	14.1	8.7	22.8
Total fat intake (g/day)								
Q1 (<49)	215	10.6	6.1	18.0	266	16.5	9.6	29.8
Q2 (49-71)	234	12.4	7.5	18.7	247	14.7	8.7	22.6
Q3 (72-103)	223	12.3	7.0	22.4	258	14.5	8.9	23.2
Q4 (>103)	278	11.8	7.3	18.4	203	14.4	8.8	23.2

Table 3.1 (continued). Descriptive statistics for unadjusted serum adiponectin levels (ug/ml) characteristics among 1,992 women in the Southern Community Cohort Study, 2002-2006

	Black women				White women			
	N	Geometric Mean	25th-75th percentile		N	Geometric Mean	25th-75th percentile	
Carbohydrate intake (g/day)								
Q1 (<173)	208	11.3	6.4	18.8	273	16.0	9.4	27.5
Q2 (173-240)	226	12.6	7.7	21.6	255	15.7	9.1	27.5
Q3 (241-335)	227	11.5	6.5	18.7	254	14.1	8.5	22.1
Q4 (>335)	289	11.7	7.5	18.5	192	14.1	8.8	22.8
Protein intake (g/day)								
Q1 (<49)	221	11.0	6.1	18.2	260	15.8	8.7	27.6
Q2 (49-70)	227	11.4	6.8	17.9	254	14.7	8.8	23.2
Q3 (71-100)	234	12.9	7.3	23.1	247	15.7	9.7	26.0
Q4 (>100)	268	11.9	7.4	18.6	213	13.8	8.7	22.0
Fiber intake (g/day)								
Q1 (<11)	220	11.2	6.8	17.6	261	14.9	8.3	24.1
Q2 (11-16)	227	11.6	6.9	19.9	254	14.7	9.2	24.3
Q3 (17-24)	229	12.6	7.0	20.5	252	16.1	9.5	28.8
Q4 (>24)	274	11.8	7.3	18.7	207	14.4	8.9	22.2
Alcohol consumption (drinks per day)								
0	496	11.8	7.1	19.7	591	14.5	8.6	23.5
1 - < 2	386	11.9	7.0	19.1	366	15.6	9.4	25.3
2+	111	11.1	6.4	18.7	37	20.9	13.2	36.5
Duration of cigarette smoking (years)								
Never smoker	414	11.2	6.8	18.0	328	15.3	8.8	25.6
<20	133	11.9	6.6	19.3	121	15.4	9.8	25.1
20-29	236	11.8	7.1	18.3	248	14.6	9.3	22.6
30+	209	12.8	7.2	22.5	293	15.2	8.9	26.2

Table 3.1 (continued). Descriptive statistics for unadjusted serum adiponectin levels (ug/ml) characteristics among 1,992 women in the Southern Community Cohort Study, 2002-2006

	Black women				White women			
	N	Geometric Mean	25th-75th percentile		N	Geometric Mean	25th-75th percentile	
Menopausal status								
Pre-	499	11.0	6.5	17.6	497	13.5	8.4	21.6
Post-	497	12.5	7.5	20.4	499	16.8	10.3	28.6
Number of live births								
None	101	13.1	7.2	22.8	96	14.5	9.1	28.6
1-2	332	12.5	7.2	20.6	477	15.0	8.7	23.2
3-4	358	11.0	6.5	18.4	323	15.4	9.6	25.2
5+	205	11.3	7.1	16.7	99	15.1	8.9	25.1
Age at menarche (years)								
<12	186	11.0	6.6	18.2	217	13.5	8.3	22.1
12	258	11.8	7.0	19.6	277	14.1	8.6	22.5
13	219	11.6	6.6	19.2	255	16.5	10.3	27.2
14	131	13.4	8.8	20.0	93	17.4	8.9	33.0
15+	197	11.5	7.0	19.3	147	15.9	9.6	24.3
Diabetes[1]								
Yes	220	11.5	6.2	19.8	164	13.4	7.5	22.1
No	776	11.8	7.1	19.0	832	15.4	9.3	25.1
Heart attack or coronary bypass surgery[1]								
Yes	41	13.9	8.6	23.8	51	15.0	9.8	21.5
No	955	11.6	6.9	18.9	945	15.1	9.0	25.0
Hypertension[1]								
Yes	600	11.3	6.5	18.7	462	13.8	7.9	22.5
No	396	12.4	7.7	19.7	534	16.3	9.9	25.5

Table 3.1 (continued). Descriptive statistics for unadjusted serum adiponectin levels (ug/ml) characteristics among 1,992 women in the Southern Community Cohort Study, 2002-2006

	Black women				White women			
	N	**Geometric Mean**	**25th-75th percentile**		**N**	**Geometric Mean**	**25th-75th percentile**	
High cholesterol[1]								
Yes	280	11.5	6.8	18.8	371	14.1	8.6	22.1
No	715	11.8	7.0	19.1	624	15.7	9.3	25.6
Depression[1]								
Yes	214	11.4	6.5	18.0	479	14.7	9.0	23.1
No	782	11.8	7.1	19.4	515	15.5	9.1	25.4

[1] Has a doctor ever told you that you have….?

Table 3.2. Linear regression results from prediction models for log-adiponectin (ug/ml) in black and white women

	Black women				White women			
Predictor	**Beta**	**Std err**	**p-value**	**Partial R^2**	**Beta**	**Std err**	**p-value**	**Partial R^2**
Body Mass Index (kg/m^2)	-0.017	0.0036	<0.0001	0.05	-0.029	0.003	<0.0001	0.11
HDL-cholesterol (mg/dl)				0.10				0.10
Q1 (<43)	Referent				Referent			
Q2 (43-50)	0.17	0.067	0.01		0.29	0.053	<0.0001	
Q3 (51-60)	0.53	0.067	<0.0001		0.41	0.057	<0.0001	
Q4 (>60)	0.59	0.064	<0.0001		0.67	0.063	<0.0001	
Age at interview (years)	0.010	0.003	<0.0001	0.01	0.015	0.003	<0.0001	0.02
Hypertension	-0.085	0.046	0.07	0.003	-0.072	0.044	0.10	0.002
Adjusted model R^2				*0.17*				*0.27*

Note: . Model estimates for the intercept and sample selection term are not shown.

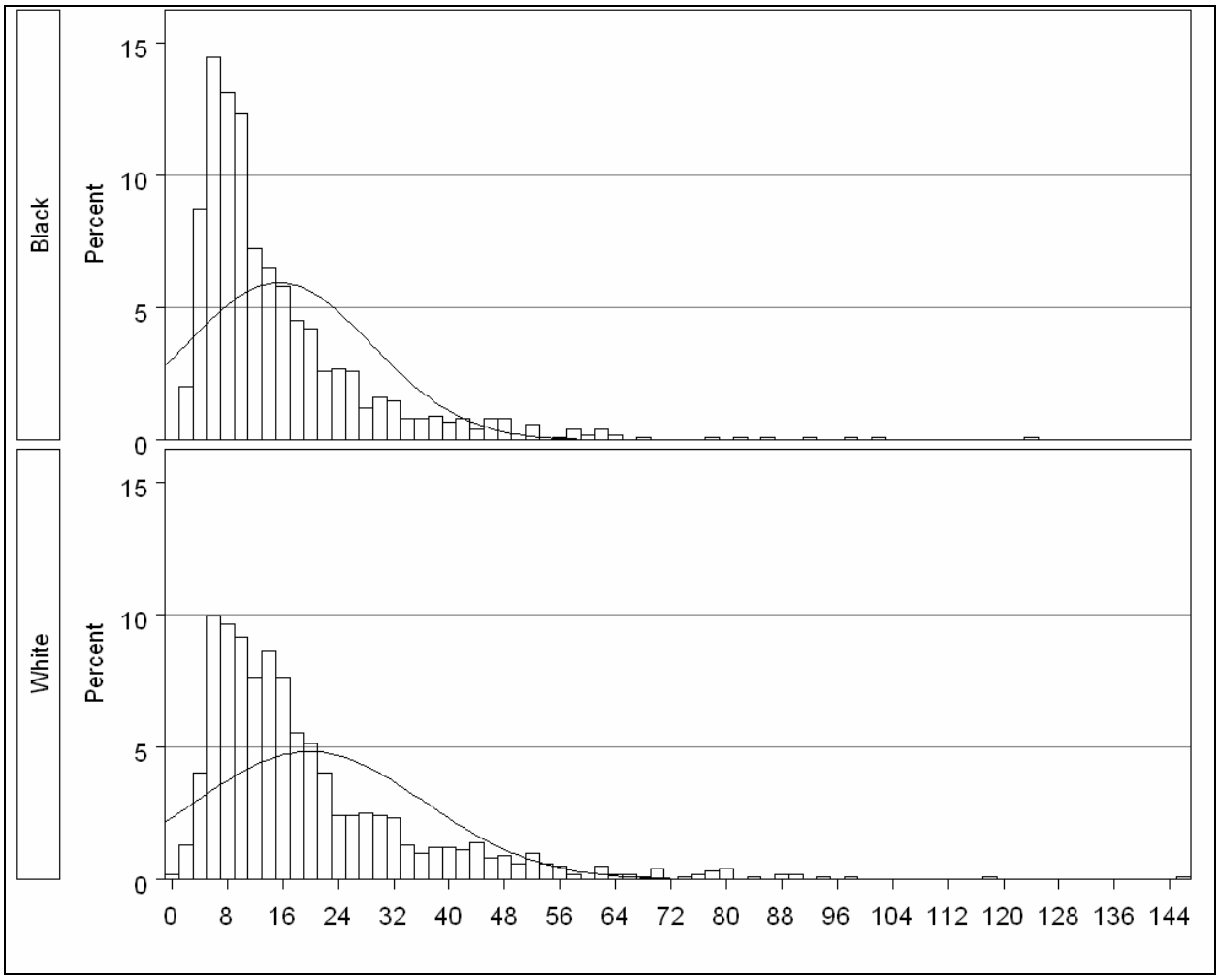

Figure 3.1. Distribution of crude adiponectin levels (ug/ml) in 996 black and 996 white women

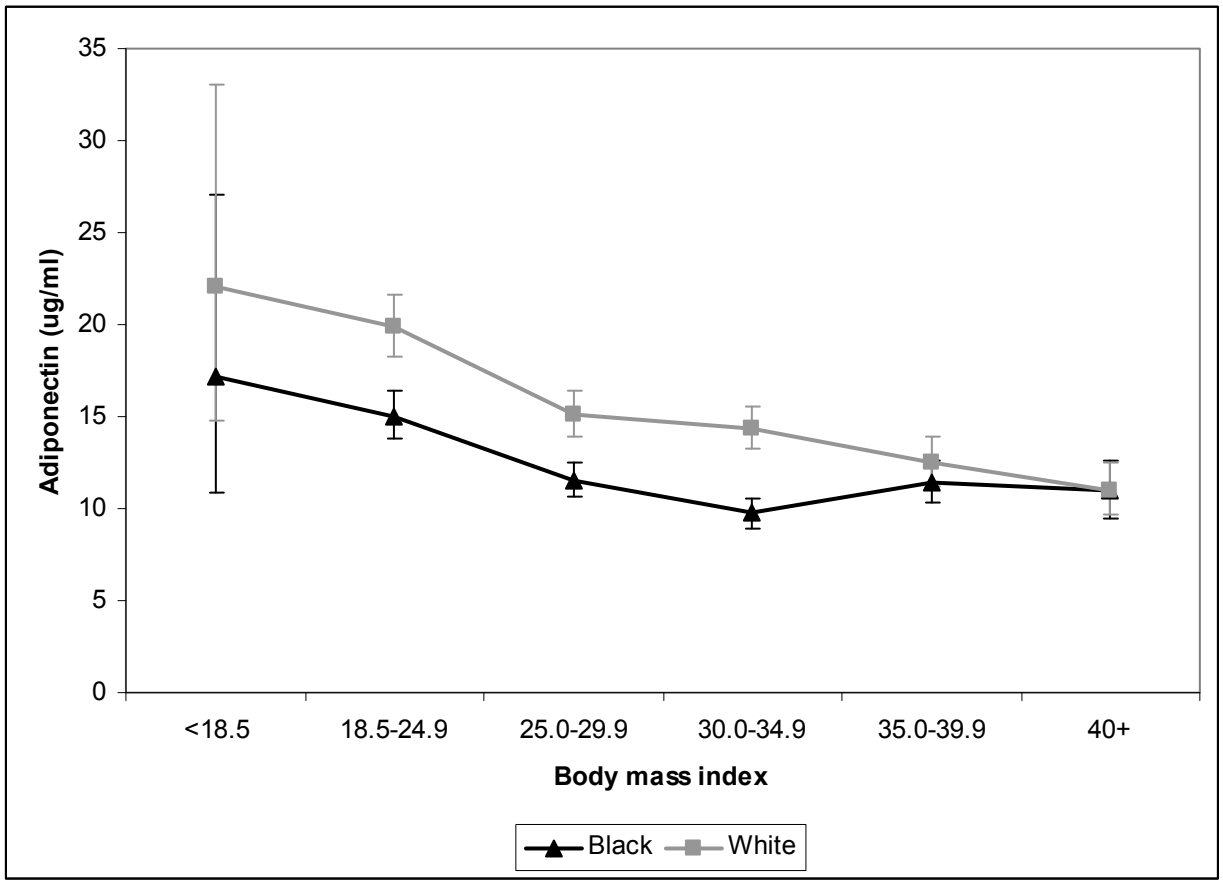

Figure 3.2. Adjusted geometric means and 95% confidence intervals for adiponectin by body mass index categories in 1,992 black and white women (adjustment factors include age, sample selection, and HDL-cholesterol).

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CHAPTER 4: *ADIPOQ*, *ADIPOR1*, AND *ADIPOR2* POLYMORPHISMS IN RELATION TO SERUM ADIPONECTIN LEVELS AND BODY MASS INDEX IN BLACK AND WHITE WOMEN

Abstract

Adiponectin is an adipose-secreted protein with suspected influence on insulin sensitivity, inflammation, and atherogenesis. Adiponectin levels are highly heritable and a number of single nucleotide polymorphisms (SNPs) in the genes encoding adiponectin (*ADIPOQ*) and its receptors (*ADIPOR1* and *ADIPOR2*) have been examined in relation to circulating adiponectin levels and obesity phenotypes. Despite differences in adiponectin levels and obesity prevalence by race, few studies of these adiponectin-related genes have included black individuals. Using cross-sectional interview data and blood samples collected from 1,967 women (977 black and 990 white) enrolled in the Southern Community Cohort Study from 2002 to 2006, we examined 25 tag-SNPs in *ADIPOQ,* 19 in *ADIPOR1*, and 27 in *ADIPOR2* in relation to serum adiponectin levels and body mass index (BMI). Associations were evaluated using race-stratified linear regression models with adjustment for age and percentage of African ancestry to account for possible population stratification. Using race-specific Bonferroni p-value thresholds for significance testing, one SNP in *ADIPOQ* was found to be significantly associated with serum adiponectin levels in white, but not black, women. Adiponectin levels (ug/ml) among white women for SNP rs17366568 were 9.3 for the A/A genotype, 13.7 for A/G, and 15.9 for G/G, p=0.00036. No other SNPs were associated with adiponectin or BMI among blacks or whites. We confirmed a recent association from two genome-wide association analyses between rs17366568 and

adiponectin in whites, and further, we are the first to report a lack of association for this SNP in a large population of black women. Because adiponectin is highly heritable and varies by race, but significant associations with polymorphisms in *ADIPOQ, ADIPOR1,* and *ADIPOR2* have been few in this and other studies, future work including large numbers of individuals of several clearly-defined race groups is needed to detect additional genetic variants that affect adiponectin and BMI levels.

Introduction

Adiponectin is a collagen-like protein produced exclusively in adipose tissue and found in relatively high concentration in serum (1). Adiponectin plays an important role in several physiological pathways including those related to insulin action, inflammation, and atherogenesis (1, 2). Adiponectin levels are inversely associated with adiposity (1, 3) and show variation by gender and race with lower levels in women compared to men (4) and in blacks compared to whites (5).

Adiponectin is encoded by the gene *ADIPOQ*, located on chromosome 3q27. Two adiponectin receptors have been identified and are encoded by the genes *ADIPOR1* (located on chromosome 1q32) and *ADIPOR2* (located on chromosome 12q13). Heritability estimates for adiponectin are high (ranging from 30% to 70%) (6, 7) and thus the genes encoding adiponectin and its receptors are plausible candidates for association with serum levels of the protein. Additionally, because adiponectin is strongly associated with body size, the adiponectin-related genes are also reasonable candidate genes for obesity. Single nucleotide polymorphisms (SNPs) in *ADIPOQ, ADIPOR1* and *ADIPOR2* have been examined in association with adiponectin levels and obesity phenotypes in several studies but results have been quite inconsistent (6). Further, virtually no studies of these genes have included black participants despite known racial differences in the prevalence of obesity (8)

and evidence showing differences in adiponectin levels by race. Thus, the goal of this study was to examine polymorphisms in *ADIPOQ*, *ADIPOR1* and *ADIPOR2* in relation to adiponectin levels and body mass index (BMI) in a large sample of both black and white women.

Methods

Institutional Review Boards at Vanderbilt University, Meharry Medical College, and the University of North Carolina at Chapel Hill approved this study.

Study population

The Southern Community Cohort Study (SCCS) is a prospective epidemiologic cohort study designed to examine racial disparities in cancer incidence and mortality (9). Study enrollment began in 2002 in twelve southeastern states at Community Health Centers (CHC) which are government-funded facilities providing health services primarily to low-income individuals in medically underserved areas. As described previously (9), participants were required to be age 40-79 years of age, English-speaking, and not have undergone treatment for cancer within the past year. From over 47,000 participants enrolled through early 2006, a sub-sample of 2,000 women who provided a blood sample at study enrollment and self-reported their race as either 'Black/African American' or 'White' was selected for further biomarker analyses. This included a random sample of 395 women selected in 2005 within strata of race, BMI, and smoking cigarette status, and a second random sample of 1,605 women selected in 2006 in equal numbers across race, BMI, and menopausal status categories.

Data collection

Trained study interviewers conducted structured baseline interviews with participants using a computer-assisted interview which elicited information including demographics,

anthropometrics, and several aspects of health and behavior. Height and weight at the time of the baseline interview were self-reported by participants and used to calculate BMI as [weight (kg)] / [height $(m)^2$]. For the 20% of women who were patients in the CHC on the day of the baseline interview, measured height and weight were abstracted from medical records for validation purposes.

A convenience blood sample was collected at the time of recruitment using one EDTA-containing plasma tube and one serum BD Vacutainer® tube. For this study, the median time between the last reported meal and blood collection was 6.0 hours for blacks and 6.3 hours for whites (p=0.07). Fasting blood, defined as at least 8 hours since last meal, was collected for 44% of the participants. Blood samples were shipped cold to Vanderbilt University in Nashville, TN, where they were processed for storage at -80° C. 84% of the blood samples were received the day after the blood draw and 98% were received within two days. The samples were frozen for an average of 2.6 years (range 3 months to 5 years) prior to analysis.

Adiponectin measurement

Adiponectin was measured in serum by immunoassay using the LINCOplex kit (Luminex® xMAP™ Technology, St. Louis, MO) in the Vanderbilt Hormone Assay and Analytical Services Core Laboratory in duplicate for each woman. The average of the two measurements was used in all analyses. Duplicate sets of samples for five randomly selected women as well as five repeat samples from each of two pooled samples were measured to assess the reliability and validity of the assay. Adiponectin was successfully measured in 1,992 of the 2,000 samples (eight samples failed due to a filter plate error or low sample volume). The intra-assay coefficient of variation was 9.4%.

Genotyping of SNPs

Candidate genes were selected for genotyping based on their relevance to cancer-related pathways and obesity, including the genes encoding for adiponectin and its receptors, *ADIPOQ, ADIPOR1,* and *ADIPOR2*. SNP selection was based on a haplotype tagging approach to select SNPs that represent common variation in the genes of interest. The International HapMap project was the primary data source for the selection of the SNPs (10). Using the Tagger pairwise method implemented in Haploview, tag-SNPs were selected based on a minor allele frequency (MAF) greater than 5% in either the Caucasian (CEU) or West African (Yoruban, YRI) populations and an r^2 cut-off of 0.8 (where r^2 is a measure of the correlation between alleles at two markers) as determined from the HapMap Project. All SNPs were scored for their ability to perform well on the Illumina GoldenGate genotyping platform using an Illumina in-house algorithm (Illumina Inc., San Diego, CA). Twenty-five SNPs were selected for *ADIPOQ*, 19 for *ADIPOR1*, and 27 for *ADIPOR2*.

An additional 292 SNPs were selected as ancestry informative markers (AIM) (11). AIMs were required to pass the Illumina scoring algorithm, be at least 5 Mb from the candidate genes, have a MAF > 0.05 in both the CEU and YRI populations, and have an allele frequency difference between the CEU and YRI populations > 0.6.

Genomic DNA was extracted from buffy coat using Puregene's DNA Purification kits (Gentra Systems, Minneapolis, MN) or Qiagen's DNA Purification kits (Qiagen, Valencia, CA) according to manufacturers' instructions. Genotyping took place at Vanderbilt University. The SNPs included in this project were grouped with those from a larger study of obesity to facilitate the use of the Illumina GoldenGate genotyping platform (Illumina Inc., San Diego, CA). Blinded QC samples (N=29) and another 171 pairs of duplicated samples were included and the consistency rate was 99.9%.

Statistical Methods

Of the 2,000 women selected for analysis, genotyping was successful for 1,990. Individuals were assigned admixture estimates (called ancestry allelic clusters or AAC) using the 292 genotyped AIMs and STRUCTURE Version 2.2.3 software (12). Given that the participants in this project were selected for inclusion based on self-reported race being only black or white (and the number of Asian and Hispanic participants in the SCCS overall is very low), the number of ancestral populations to be estimated was *a priori* specified to be 2. Thus two AACs were generated for each individual: one for African ancestry and one for European ancestry. Twenty-three women were excluded from analysis because of discrepancies between self-reported race and ancestry estimates derived from STRUCTURE leaving 1,967 women for study (N=990 black and N=977 white). For analyses with adiponectin as the outcome, 1,959 women were examined after excluding eight for whom adiponectin could not be measured.

We tested for Hardy-Weinberg equilibrium in race-stratified samples and found one SNP in *ADIPOQ* (rs1648707) that showed significant deviation from Hardy-Weinberg equilibrium in both white ($p=8.6x10^{-16}$) and black ($p=1.3x10^{-16}$) women. This SNP was excluded from all further analyses.

Linkage disequilibrium was calculated and displayed between each of the genotyped SNPs, stratified by race, using the r^2 metric and Haploview software (Supplementary Figure 4.1).

Associations between individual SNPs and adiponectin levels were examined using race-stratified multiple linear regression models with log-transformed adiponectin as the outcome. Similar models were constructed with BMI as the outcome variable. SNPs were generally examined using a codominant inheritance model (with 2 degree of freedom) with

the referent genotype selected to be the most common race-specific homozygous genotype. For SNPs in which less than 10 women had the rare homozygous genotype, a dominant model was used that combined women with the rare homozygous genotype with those with the heterozygous genotype. Each regression model included adjustment for age at baseline SCCS interview as well as percentage of African ancestry as estimated by STRUCTURE. Adjusted mean adiponectin levels for each genotype were back-transformed for presentation. Models including adjustment for the first five principal components derived using EIGENSTRAT software (13) in place of the AAC derived from STRUCTURE were also examined and found to have very similar results. SAS version 9.2 (SAS Institute) was used for all modeling.

A Bonferroni correction was applied to the *a priori* alpha level of 0.05 and was calculated based on examination of individual models for each SNP over the three genes of interest. Alpha values of 0.00096 and 0.00075 were used for white and black women, respectively, based on the analysis of 52 and 67 SNPs.

Results

Consistent with the stratified sampling design for this study, equal numbers of black and white women were post-menopausal, and women in both race groups had a mean BMI (BMI) of approximately 30 kg/m^2 (Table 4.1). Income and education distributions between the race groups were also similar. Adiponectin levels were lower in black women than in white women (15.4 v 19.9, mg/ml). There were no differences in adiponectin levels by fasting blood status (black fasting v. non-fasting mean=14.9 v. 15.9, p=0.25; white fasting v. non-fasting mean=20.2 v. 19.6, p=0.6).

The location and genotype frequencies of the SNPs selected for the three genes of interest are described in Tables 4.2a, 4.2b, and 4.2c. Genotyping was successful for all 1,967

women for 58 (84%) of the SNPs and over 96% complete for the other 11 SNPs. More tag-SNPs were needed to provide adequate gene coverage for the black sample compared to the white sample. SNPs that were found to have a MAF < 0.001 within a race group were omitted from further analyses in that race group.

Adjusted mean adiponectin levels by genotype are shown by race in Figure 4.1 for *ADIPOQ*, *ADIPOR1*, and *ADIPOR2*. One SNP, rs17366568 in white women (p=0.00036), met the Bonferroni p-value threshold for significance. This SNP was not in LD with any of the other genotyped SNPs and thus haplotype analyses were not conducted (Supplementary Table 4.1). Otherwise, we observed no significant associations between adiponectin levels and individual SNPs. Figure 4.2 shows adjusted mean BMI values by genotype for the three genes of interest. There was little variation by BMI across genotypes in any of the SNPs and none met the Bonferroni p-value threshold for significance.

For the single SNP meeting the Bonferroni p-value threshold (rs17366568 in *ADIPOQ* in white women), the additive genetic model was also examined. rs17366568 was found to be significantly associated with adiponectin using this model form with little difference seen compared to the co-dominant model (additive model p-value=0.0002 versus p=0.00036 for co-dominant model). BMI was added as a covariate to the linear regression model for rs17366568 in *ADIPOQ* in relation to adiponectin levels, and while it was found to be a very strongly associated with adiponectin itself, its inclusion did not alter the association between the SNP and the adiponectin levels (data not shown).

Interactions between SNP rs17366568 and BMI, diabetes status, and menopausal status were examined. Of these factors, only BMI was found to be a significant effect modifier of the SNP-adiponectin association (likelihood ratio p-value < 0.0001 for

interaction). A strong positive association was seen for adiponectin levels among individuals of non-obese BMI (<25 kg/m^2) (adiponectin=8.1, 19.3, and 23.7 ug/ml for A/A, A/G, and G/G genotypes, respectively; p=0.004); this marked increase in adiponectin levels across genotype was not as clear in the other strata of BMI (25-29, 30-34, and 35+) (Figure 4.3). However, these interaction models should be considered exploratory as the number of individuals in some cells was very small (for example, N=3 for individuals with BMI <25 kg/m^2 and genotype A/A).

The percentage of African ancestry covariate was found to be non-significant in white women but borderline significant in black women (p=0.1) for SNP rs17366568 in *ADIPOQ*. Race-combined models were also examined for each SNP-outcome association in order to increase statistical power to detect associations. In these models adjusted for age and percentage of African ancestry, SNP rs17366568 in *ADIPOQ* remained significantly associated with adiponectin and no additional SNPs were found to meet the Bonferroni corrected p-value for statistical significance in relation to either adiponectin or BMI.

Discussion

This study examined effects of variation in the adiponectin-related genes on adiponectin levels and BMI in a large, population-based sample using a pathway-based approach. We observed a significant association between adiponectin levels and SNP rs17366568 in *ADIPOQ* among white women, a finding also recently reported by two recent genome-wide association studies (GWAS) of European whites (7, 14). This SNP was not found to be associated with adiponectin levels among black women in our study which was the first, to our knowledge, to examine the adiponectin-related genes in blacks. Determining relevant SNPs affecting adiponectin levels and BMI, and whether these SNPs differ across racial groups, is an important step in our understanding of the roles played by adiponectin and

obesity in the complex mechanisms underlying racial disparities for major chronic disease such as diabetes, cardiovascular disease, and many cancers.

Our pathway-based results for SNP rs17366568 in *ADIPOQ* were very similar to those seen in the two GWAS studies (7, 14) that also found significant associations with this SNP and adiponectin levels. The A/A genotype was relatively rare in our study (MAF=0.14, N=19 white women had the A/A genotype) as it was in the two GWAS (MAF=0.11 and 0.13) (7, 14). The effect size (using log-transformed adiponectin, ug/ml, as the outcome) for an additive model was 0.20 for each addition of the G allele in the KORA portion of the Heid study (7), which was very close to our effect estimate of 0.18 for a similar model. The proportion of variance explained by this SNP was 1.7% in our pathway-based study, slightly lower than the 5.3% reported for women in one GWAS (7) but close to the <2% reported in the other GWAS (14)

In our study, we found no additional SNPs beyond rs17366568 that were associated with adiponectin in black or white women. Adiponectin levels have been examined in relation to variants in *ADIPOQ* in more than a dozen association studies of white and Asian individuals but few specific SNPs or haplotypes have been replicated in multiple populations. Four common *ADIPOQ* polymorphisms (rs2241766 (commonly called 45T→G), rs1501299 (commonly called 276G→T), -11391G→G, and -11377C→G) were genotyped in early candidate gene studies for association with adiponectin with inconsistent results (15-25). Several of these studies were examined in a 2007 meta-analysis of genetic variants in *ADIPOQ* in relation to adiponectin levels. Menzaghi et al. reported that two of the most commonly typed SNPs on *ADIPOQ*, rs17300539 and rs1501299, were significantly associated with adiponectin levels in the meta-analysis of five and twelve studies,

respectively (6). Neither of these SNPs was genotyped in our study but each was in strong linkage disequilibrium (LD) with selected tag SNPs that we did genotype. In the HapMAP CEU population (release 22), both rs822387 and rs16861210 had a pairwise r^2 value of 0.82 with rs17300539, but neither was found to be significantly associated with adiponectin levels in white women in our study (p=0.2 for rs822387 and p=0.1 for rs16861210). SNP rs6444175 in *ADIPOQ* has a pairwise r^2 value of 0.92 in the CEU population and an r^2 of 0.53 in the YRI population with rs1501299, but, again, neither of these SNPs was found to be significantly associated with adiponectin levels in our study (p=0.5 for white women and p=0.6 for black women). As genotyping larger numbers of SNPs has become easier and more cost-efficient, additional SNPs in *ADIPOQ* have been typed and examined in relation to adiponectin levels but still little consistency has been observed in association with adiponectin across studies (4, 26-29).

Regarding BMI, several studies have reported positive associations between BMI and *ADIPOQ* variants (30-33) while others have not found any significant effects (16, 20, 25, 28, 34). No significant associations with BMI were found with SNPs in *ADIPOQ* in the Menzaghi 2007 meta-analysis (6). Positive associations in past reports were found for several different SNPs in *ADIPOQ* with little replication across studies. Only rs182052, found to be associated with BMI in a group of over 800 Hispanic individuals (32), was also genotyped in our study but we did not find any association with BMI in black (p=0.95) or white women (p=0.14).

Very few studies have examined polymorphisms in *ADIPOR1* and *ADIPOR2* in relation to adiponectin or BMI. No genome-wide significant associations between adiponectin levels and SNPs in either *ADIPOR1* or *ADIPOR2* were found in a GWAS of

Europeans (14). Loos et al. investigated two SNPs in *ADIPOR1* (rs1539355 and rs2275737) and two in *ADIPOR2* (rs0773982 and rs2058112) in relation to BMI in French-Canadians and found no statistically significant associations (25); we also observed no association with BMI for these four SNPs in black or white women.

High heritability estimates for both adiponectin levels and obesity as well as linkage studies showing the adiponectin-related genes to be strongly related to these phenotypes indicate that while the data have been inconsistent, polymorphisms in the genes encoding adiponectin and the two known adiponectin receptors remain promising avenues for explaining variation in adiponectin levels as well as obesity. Conflicting results to date may be due to a myriad of differences across studies including sample size, analysis methodology (including the examination of single-SNPs versus haplotypes, differing approaches to confounding, control for population stratification, and multiple comparisons), genetic background, and possibly environmental or gene-gene influences across populations. Studies of other adipokines in relation to genetic variation in the protein-encoding or receptor-encoding genes have been similarly inconsistent. For leptin, perhaps the best studied of the adipokines, relatively scant data are available examining polymorphisms in the *LEP* or *LEPR* genes in relation to circulating leptin levels and conflicting evidence exists for associations with specific SNPs (35-38).

It remains possible that there are unknown rare variants that have a strong effect on adiponectin levels, and few studies to date, including ours, have been well-powered to detect rare variants. There may also be many as-yet unidentified common loci with small individual effects on adiponectin levels. Additionally, genetic contribution to adiponectin variation may be influenced by interactions between multiple loci or between loci and

environmental factors or perhaps by epigenetic factors, none of which have been carefully examined yet.

Individuals of African descent display, on average, more variation in allele frequency than do people of European descent, indicating that the frequency of etiologically important SNPs may differ by race. The difference in the association with adiponectin levels and SNP rs17366568 between black and white women seems at least in part due to allele frequency differences with the MAF=0.14 for the white women and only 0.015 for black women. This is consistent with the results of the HapMap project in which all of the YRI samples were found to have the G/G genotype (and thus this SNP is not shown on the LD plot for the HapMap YRI population, Figure 4.4, top panel).

LD plots for *ADIPOQ* for the HapMap YRI and CEU populations are shown in Figure 4.4 (top and bottom panels, respectively) and demonstrate the importance of examining genetic correlates of adiponectin and obesity in racially diverse populations. However, to date, polymorphisms in *ADIPOQ, ADIPOR1*, and *ADIPOR2* have been examined almost exclusively in populations that did not include individuals of African descent. One genome-wide linkage scan which included both 89 Hispanic families and 42 black families reported heritability estimates of 71% and 64% in Hispanics and blacks, respectively, for adiponectin but the region of the genome where *ADIPOQ* is located was identified as a major quantitative trait loci only in the Hispanic sample (39). The HERITAGE study included 276 black participants and found two variants in *ADIPOQ* that were associated with measures of body fat in blacks but not whites (34). Beyond these two reports with only limited numbers of black participants, our study is the first to examine

either adiponectin levels or BMI in relation to adiponectin-related genes in a large group of comparable black and white participants.

The major strength of this study was the use of participants from the SCCS which allowed for the examination of adiponectin levels and BMI in relation to genetic variation in adiponectin-related genes in a large, population-based sample of black and white women. SNPs were selected to provide coverage across the genes using both the HapMap CEU and YRI populations which resulted in similar precision in estimating sequence variation in both the black and white participants. Study limitations include the potential for measurement error in either of the outcome variables of adiponectin and BMI. As is common in large population-based studies, we measured total adiponectin rather than high-molecular weight adiponectin. Additionally, we only measured adiponectin at one point in time; however, adiponectin levels measured one year apart have been reported to be highly correlated and likely sufficient for large epidemiologic studies (40, 41). It has also been suggested that serum adiponectin levels, as we and others have measured, may not reflect the overall amount of adiponectin in the body or adiponectin concentrations in areas that are targets for this protein but much work remains to determine how to more meaningfully measure adiponectin (6). We also did not require participants to provide fasting blood samples although we found no differences in the mean adiponectin levels by fasting status. For BMI, we used self-reported height and weight measures to calculate BMI. While there is evidence to indicate that women tend to over-report height while under-reporting weight (42), BMI values calculated from self-reported height and weight in the SCCS were very highly correlated with BMI values calculated from medical record data overall (Pearson correlation coefficient > 0.95) as well as across strata of race and BMI, indicating that the self-reported

values are generally of good quality. A further limitation of this study is the lack of information regarding central obesity which may be a stronger correlate of disease risk than BMI and has been observed to vary across race groups for a given BMI (43).

In this first-ever study of genetic variation in adiponectin-related genes in relation to adiponectin and BMI among both black and white women, we demonstrated that there may be different genetic variants that contribute to variation in adiponectin levels by race. We confirmed an association observed recently in two GWAS between SNP rs17366568 located in *ADIPOQ* and adiponectin levels in white women, but we did not find any association with this SNP in black women. Additionally, we found no other SNPs that were associated with adiponectin levels or with BMI in black or white women. Future discovery of additional variants that affect adiponectin levels (and particularly rare variants that may occur only in certain race groups) as well as detection of gene-gene and gene-environment interactions related to adiponectin levels and BMI will require future studies with large sample sizes from multiple racial groups.

Acknowledgements

This project was funded in part by grant OP05-0927-DR1 from Susan G. Komen for the Cure. The Southern Community Cohort Study is funded by grant R01 CA92447 from the National Cancer Institute (NCI). Ms. Cohen also received support from NCI Training Grants T32 CA09330-26 and 5-R25-CA057726. Dr. Gammon is supported in part from P30ES10126 from the National Institute of Environmental Health Sciences.

Table 4.1. Characteristics of black and white women genotyped for *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* from the Southern Community Cohort Study, 2002-2006

	Black women (N=996)		White women (N=994)	
	Mean	**[std]**	**Mean**	**[std]**
Age at baseline interview (years)	50.1	[8.9]	49.9	[8.6]
Body mass index (kg/m^2)	30.4	[6.4]	30.3	[6.6]
Adiponectin (ug/ml)	15.4	[13.4]	19.9	[16.1]
	N	**(%)**	**N**	**(%)**
Education				
<9 years	78	(7.8)	86	(8.7)
9-11 years	243	(24.4)	221	(22.2)
Completed high school	411	(41.3)	411	(41.4)
More than high school	264	(26.5)	276	(27.8)
Annual Household Income				
< $15,000	621	(62.9)	597	(60.3)
$15,000 – 24,999	233	(23.6)	199	(20.1)
$25,000 – 49,999	116	(11.8)	130	(13.1)
$50,000+	17	(1.7)	64	(6.5)
Missing	9		4	
Menopausal status				
Pre	497	(49.9)	497	(50.0)
Post	499	(50.1)	497	(50.0)
Diabetes[1]				
Yes	218	(21.9)	162	(16.3)
No	778	(78.1)	832	(83.7)

[1] Self-reported from the question "Has a doctor ever told you that you have diabetes?"

Table 4.2a. Characteristics of *ADIPOQ* SNPs genotyped in black and white women in the Southern Community Cohort Study

						Genotype frequency					
						Black women (N=990)			White women (N=977)		
SNP	Gene region	Tagging popn	Coded allele	Non-coded allele	N	C/C	C/NC	NC/NC	C/C	C/NC	NC/NC
rs864265	5' near gene	YRI, CEU	C	A	1,967	0.75	0.23	0.02	0.70	0.27	0.03
rs822387	5' near gene	YRI	A	G	1,967	0.45	0.44	0.11	0.83	0.16	0.008
rs16861194	5' near gene	YRI, CEU	A	G	1,967	0.57	0.38	0.05	0.84	0.16	0.006
rs182052	5' near gene	YRI	G	A	1,967	0.41	0.48	0.11	0.44	0.44	0.12
rs16861205	5' near gene	YRI	G	A	1,967	0.65	0.33	0.04	0.85	0.14	0.006
rs822391	Intron 1	CEU	A	G	1,967	0.92	0.08	0	0.62	0.32	0.06
rs16861210	Intron 1	YRI, CEU	G	A	1,967	0.68	0.29	0.03	0.82	0.17	0.01
rs822396	Intron 1	CEU	A	G	1,967	0.63	0.32	0.05	0.65	0.31	0.05
rs12495941	Intron 1	YRI, CEU	C	A	1,967	0.40	0.46	0.14	0.43	0.46	0.11
rs7649121	Intron 1	CEU	A	T	1,967	0.79	0.20	0.01	0.68	0.29	0.02
rs9877202	Intron 1	YRI	A	G	1,967	0.71	0.26	0.03	0.99	0.002	0
rs17366568	Intron 1	CEU	G	A	1,967	0.97	0.03	0.001	0.75	0.23	0.02
rs3821799	Intron 1	CEU	G	A	1,967	0.18	0.49	0.33	0.33	0.47	0.20
rs3774261	Intron 1	YRI, CEU	G	A	1,967	0.18	0.50	0.31	0.41	0.45	0.15
rs17366743	Intron 2	CEU	A	G	1,967	0.99	0.01	0	0.95	0.05	0.003
rs6444174	Intron 2	YRI	A	G	1,963	0.70	0.27	0.03	0.99	0.006	0
rs1063539	Exon 3	CEU	C	G	1,879	0.62	0.38	0.002	0.75	0.23	0.02
rs9842733	3' UTR	YRI	T	A	1,967	0.83	0.16	0.008	0.99	0.003	0
rs1403697	3' UTR	YRI	A	G	1,967	0.77	0.22	0.02	0.99	0.003	0
rs7641507	3’ near gene	YRI	G	A	1,967	0.85	0.15	0.005	0.99	0.004	0
rs1403696	3’ near gene	YRI	G	A	1,967	0.62	0.33	0.05	0.99	0.007	0
rs6444175	3’ near gene	YRI, CEU	G	A	1,967	0.46	0.46	0.08	0.56	0.36	0.08
rs7628649	3’ near gene	YRI, CEU	G	A	1,967	0.46	0.46	0.11	0.79	0.19	0.01
rs17373414	3’ near gene	CEU	G	A	1,967	0.98	0.02	0.001	0.78	0.20	0.02

Table 4.2b. Characteristics of *ADIPOR1* SNPs genotyped in black and white women in the Southern Community Cohort Study

						Genotype frequency					
						Black women (N=990)			White women (N=977)		
SNP	Gene region	Tagging popn	Coded allele	Non-coded allele	N	C/C	C/NC	NC/NC	C/C	C/NC	NC/NC
rs6672643	5' near gene	YRI, CEU	A	G	1965	0.56	0.37	0.07	0.75	0.22	0.03
rs2185781	5' near gene	YRI, CEU	G	A	1967	0.66	0.30	0.04	0.65	0.30	0.04
rs4336908	5' near gene	YRI	G	A	1967	0.86	0.13	0.01	0.65	0.30	0.04
rs10920531	5' near gene	YRI, CEU	C	A	1967	0.24	0.46	0.30	0.43	0.42	0.15
rs7539542	Exon 8	CEU	G	C	1967	0.16	0.44	0.40	0.49	0.40	0.10
rs1342387	Intron 4	YRI	G	A	1966	0.25	0.49	0.26	0.32	0.45	0.23
rs7518457	Intron 4	YRI	A	G	1967	0.88	0.11	0.01	0.99	0.004	0
rs12045862	Intron 3	YRI, CEU	G	A	1967	0.83	0.16	0.01	0.55	0.38	0.07
rs2275737	Intron 1	YRI, CEU	C	A	1967	0.31	0.49	0.20	0.35	0.44	0.21
rs12733285	Intron 1	CEU	G	A	1967	0.62	0.33	0.05	0.49	0.41	0.09
rs10753929	Intron 1	YRI, CEU	G	A	1967	0.65	0.31	0.04	0.76	0.21	0.02
rs1539355	Intron 1	YRI, CEU	A	G	1967	0.29	0.50	0.21	0.49	0.41	0.10
rs10800888	3’ Near gene	YRI	G	A	1967	0.76	0.23	0.01	0.99	0.002	0
rs6666089	3’ Near gene	YRI	G	A	1967	0.75	0.23	0.02	0.49	0.41	0.10
rs7523903	3’ Near gene	YRI	G	C	1963	0.59	0.40	0.01	0.98	0.02	0
rs2232849	3’ Near gene	YRI	G	A	1967	0.83	0.16	0.009	0.99	0.004	0
rs2232844	3’ Near gene	YRI	A	G	1967	0.83	0.16	0.009	1.00	0	0
rs2232842	3’ Near gene	YRI	A	G	1967	0.71	0.27	0.02	0.94	0.05	0.002

Table 4.2c. Characteristics of *ADIPOR2* SNPs genotyped in black and white women in the Southern Community Cohort Study

						Genotype frequency					
						Black women (N=990)			White women (N=977)		
SNP	Gene region	Tagging popn	Coded allele	Non-coded allele	N	C/C	C/NC	NC/NC	C/C	C/NC	NC/NC
rs758027	5' near gene	YRI	A	G	1966	0.81	0.18	0.01	0.99	0.001	0
rs1029629	5' near gene	YRI	A	C	1966	0.56	0.39	0.05	0.50	0.40	0.09
rs7304096	Intron 1	TRI	A	G	1967	0.93	0.07	0.001	0.99	0.001	0
rs2058033	Intron 1	CEU	A	C	1967	0.95	0.05	0.001	0.76	0.21	0.02
rs7975600	Intron 1	YRI, CEU	T	A	1967	0.75	0.23	0.01	0.73	0.24	0.02
rs11832817	Intron 1	CEU	G	A	1967	0.70	0.28	0.02	0.52	0.40	0.08
rs12826079	Intron 1	CEU	G	A	1966	0.98	0.02	0	0.87	0.12	0.006
rs10773982	Intron 1	YRI, CEU	A	G	1967	0.39	0.47	0.14	0.46	0.43	0.11
rs11061946	Intron 1	CEU	G	A	1966	0.98	0.02	0.001	0.86	0.13	0.004
rs10773983	Intron 1	CEU	G	A	1966	0.07	0.38	0.56	0.49	0.40	0.11
rs12316367	Intron 1	YRI	A	G	1967	0.02	0.26	0.72	0.29	0.49	0.22
rs10773989	Intron 1	YRI	A	G	1967	0.54	0.40	0.06	0.24	0.52	0.25
rs2058112	Intron 1	CEU	G	A	1967	0.62	0.33	0.05	0.75	0.24	0.01
rs12298275	Exon 2	YRI	A	G	1967	0.92	0.08	0.001	1.00	0	0
rs7134070	Intron 2	YRI	A	G	1967	0.74	0.24	0.02	0.99	0.01	0
rs7967137	Intron 2	YRI	A	G	1967	0.51	0.41	0.09	0.75	0.24	0.01
rs7138701	Intron 2	YRI	G	A	1967	0.66	0.31	0.04	0.99	0.01	0
rs11614639	Intron 3	YRI	A	C	1967	0.30	0.51	0.19	0.32	0.49	0.20
rs10773991	Intron 3	YRI	A	G	1967	0.03	0.33	0.64	0.29	0.49	0.22
rs4140993	Intron 4	YRI	A	C	1967	0.70	0.27	0.03	0.99	0.01	0
rs16928751	Exon 6	YRI, CEU	G	A	1967	0.61	0.35	0.05	0.75	0.24	0.01
rs2286384	Intron 6	YRI, CEU	C	G	1967	0.08	0.46	0.46	0.29	0.49	0.22
rs12342	3' UTR	YRI	G	A	1967	0.65	0.32	0.03	0.49	0.41	0.10
rs1044471	3' UTR	YRI, CEU	G	A	1967	0.63	0.33	0.04	0.26	0.51	0.23
rs7294540	3’ Near gene	YRI	A	C	1967	0.74	0.25	0.02	0.18	0.49	0.34
rs13219	3’ Near gene	YRI, CEU	A	G	1967	0.02	0.27	0.71	0.33	0.48	0.19
rs2058111	3’ Near gene	YRI	A	C	1963	0.65	0.32	0.03	0.19	0.48	0.34

Tables 4.2a, b, and c abbreviations:

C/C = coded allele/coded allele; C/NC = coded allele/non-coded allele; NC/NC = non-coded allele/non-coded allele
UTR = untranslated region
YRI = Yoruban population from HapMap; CEU = Caucasian population from HapMap
C/C = Common/Common genotype; C/r = Common/rare genotype; r/r = rare/rare genotype

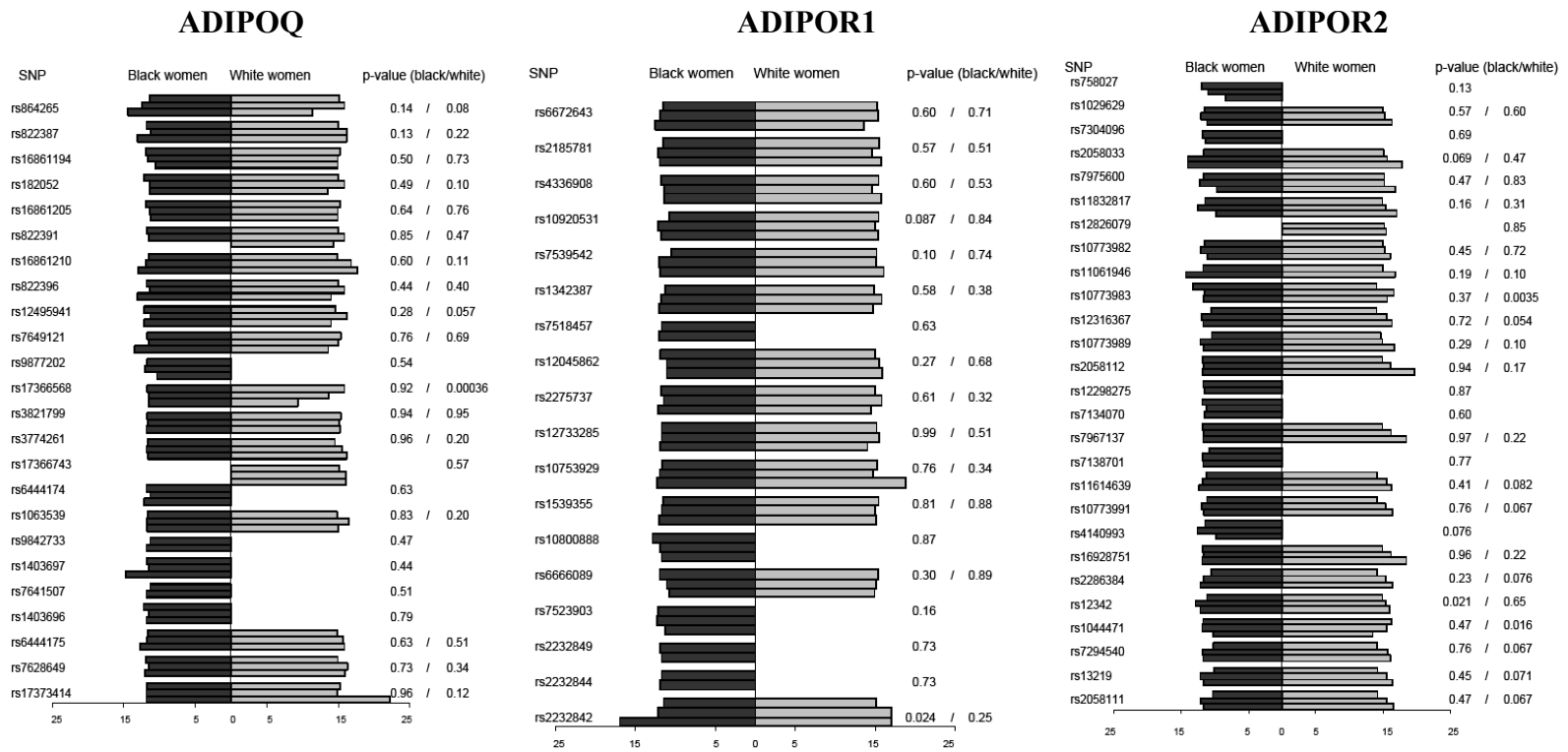


Figure 4.1. Adjusted mean adiponectin levels (ug/ml) and associated p-values from race-stratified linear regression models for SNPs in *ADIPOQ* (left panel), *ADIPOR1* (center panel), and *ADIPOR2* (right panel) among 990 black women and 977 white women, Southern Community Cohort Study, 2002-2006

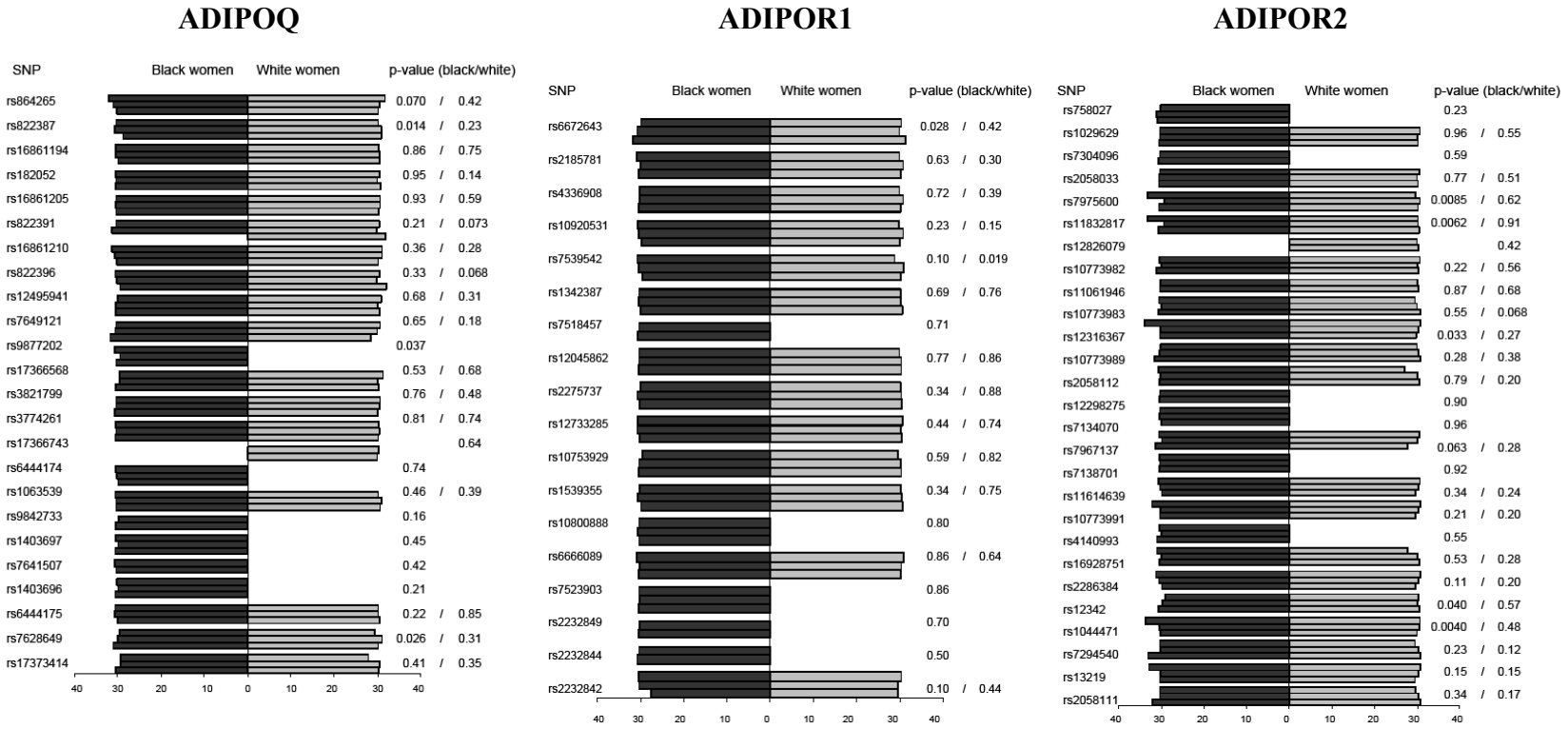


Figure 4.2. Adjusted mean body mass index (kg/m2) values and associated p-values from race-stratified linear regression models for SNPs in *ADIPOQ* (left panel), *ADIPOR1* (center panel), and *ADIPOR2 (*right panel) among 990 black women and 977 white women, Southern Community Cohort Study, 2002-2006

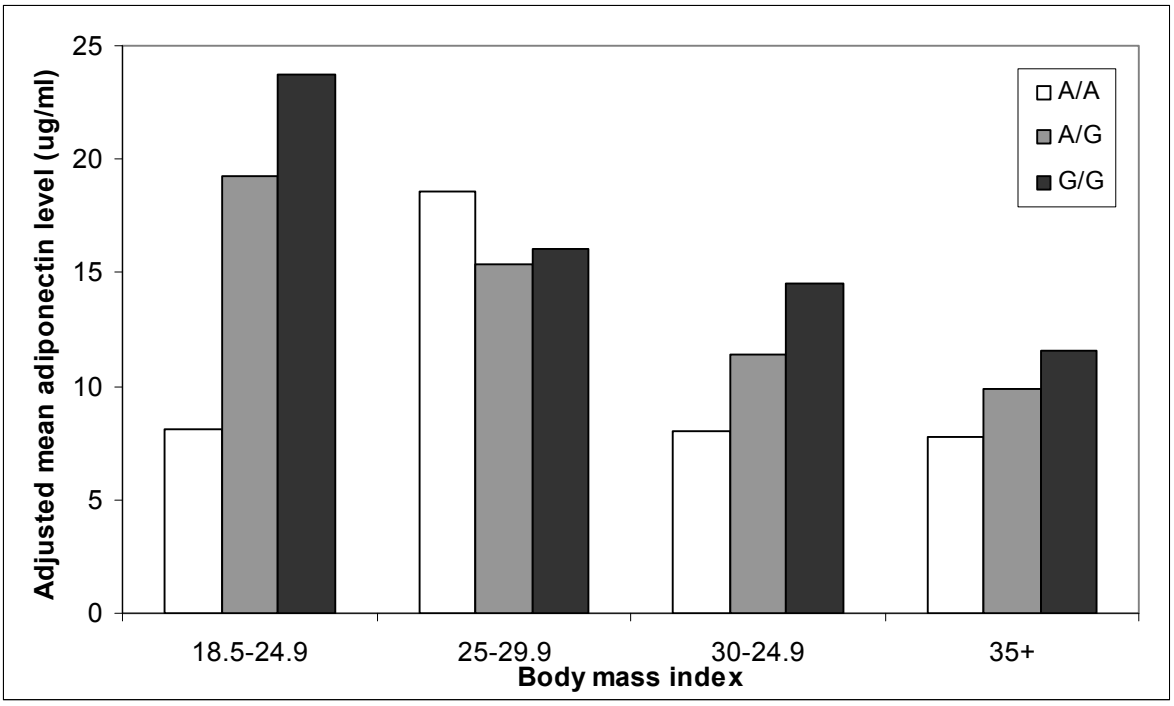


Figure 4.3. Adjusted mean adiponectin levels (ug/ml) for genotypes in SNP rs17366568 in *ADIPOQ* among white women from linear regression models stratified by body mass index

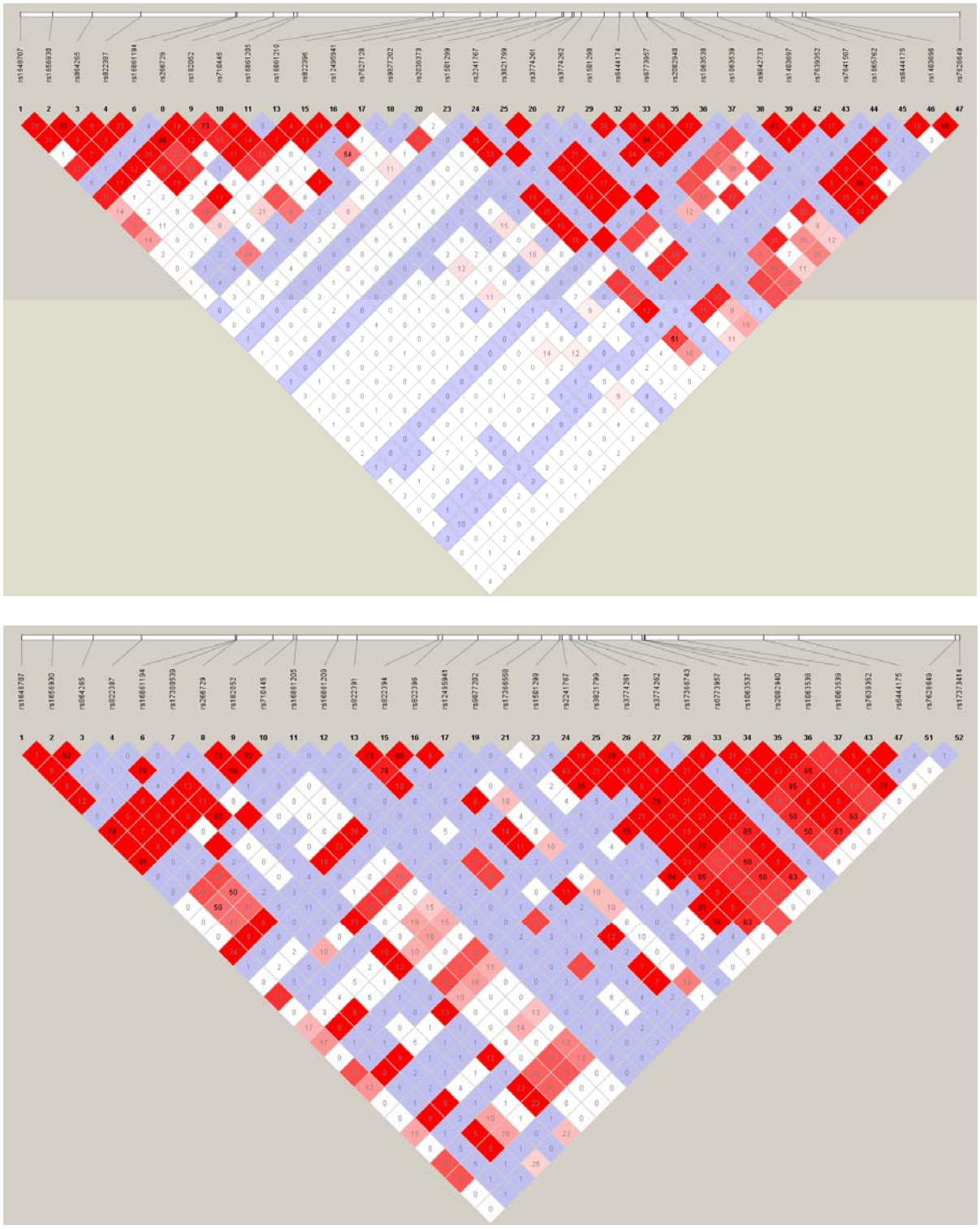
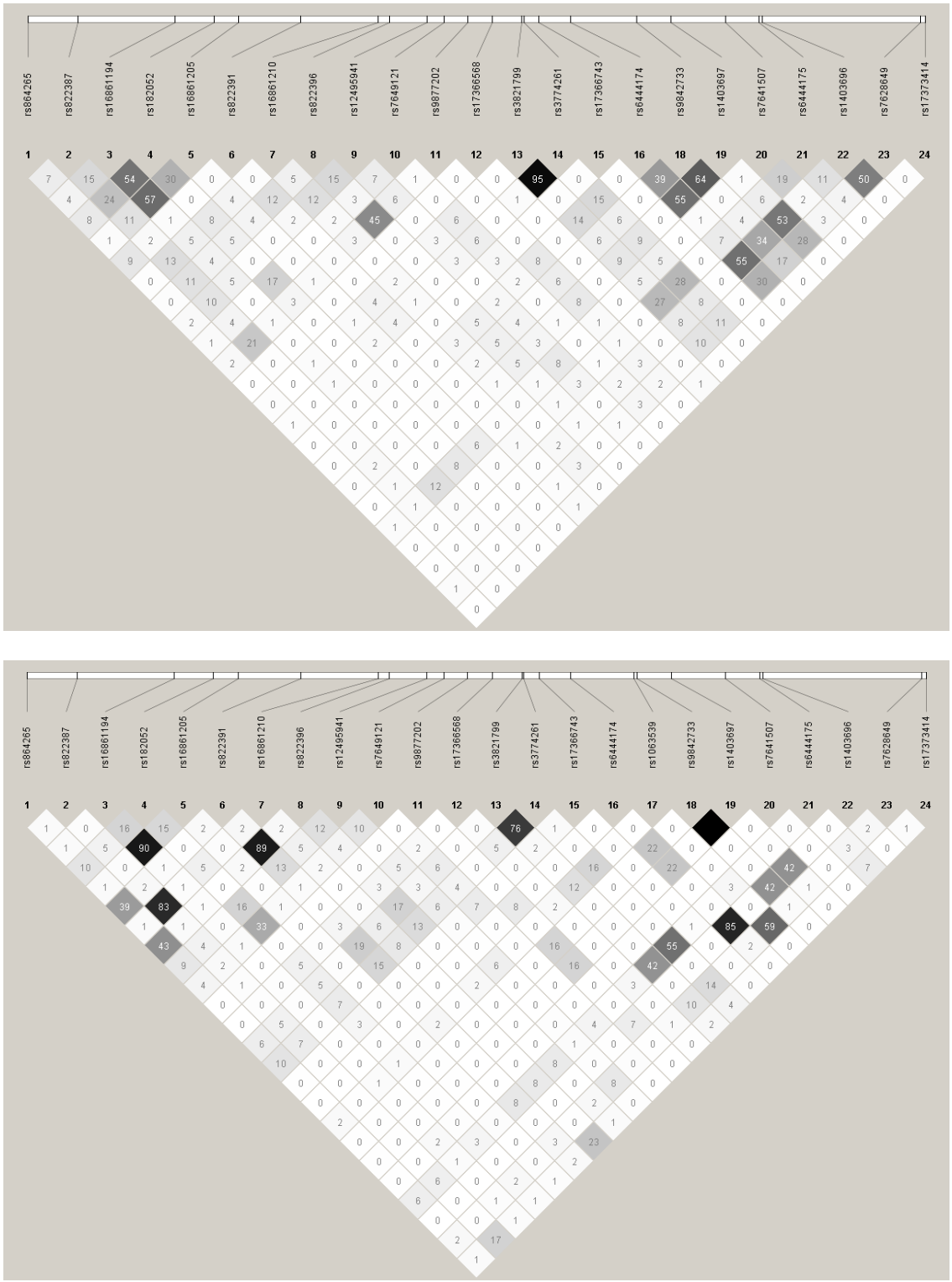


Figure 4.4. Linkage disequilibrium (LD) plots showing r2 values for the Yoruban (YRI) (top panel) and Caucasian/CEPH (CEU) (bottom panel) populations from HapMap phase II data, NCBI build 36 including the gene region for *ADIPOQ* plus 10 kb downstream and upstream**.**



Supplementary Figure 4.1. Linkage disequilibrium (LD) plots for *ADIPOQ* showing r^2 values between genotyped SNPs in 990 black women (top panel) and 977 white women (bottom panel), Southern Community Cohort Study, 2002-2006

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CHAPTER 5: DISCUSSION

Summary

Using interview data and blood samples collected at baseline from the Southern Community Cohort Study, this cross-sectional study examined serum adiponectin levels in relation to a variety of environmental and behavioral factors as well as several single nucleotide polymorphisms (SNPs) in three adiponectin-related genes (*ADIPOQ, ADIPOR1,* and *ADIPOR2*). This study was the largest to-date to measure adiponectin in both black and white women. The results showed that black women have lower adiponectin levels compared to whites even after adjustment for body mass index (BMI). These results expand upon previous studies that were limited by small sample sizes (1-4) or narrow age ranges (5-7) and demonstrate that racial differences in adiponectin exist across the spectrum of adult age and body size. In an examination of predictors of adiponectin levels, age, HDL-cholesterol, and hypertension were found to be strong correlates of adiponectin in both race groups. In the genetic analyses, one SNP (rs17366568) on *ADIPOQ* was found to be significantly associated with adiponectin levels in white women but not in black women. This finding is supported by similar observations in two recent genome-wide association studies (GWAS) in European whites which found differences in adiponectin levels across genotypes of this SNP (8, 9). Further, for the first time, this study examined SNP rs17366568 in blacks. Different results compared to white women were found for this SNP; among the black women in this study, no association was observed with rs173665368 and

adiponectin levels. No significant associations were observed between any of the SNPs in the three adiponectin-related genes and BMI.

Study Strengths

Study design

This study was a cross-sectional design using data from 2,000 women enrolled in the SCCS. From the larger SCCS population, women were randomly selected for inclusion in this study sample within strata of race, menopausal status, and BMI category. This design ensured that sufficient numbers of women in each race group were included which allowed this study to overcome problems with small sample sizes of blacks in previous studies of adiponectin across race groups. Further, by including women in equal numbers across categories of BMI, the study was amply powered to quantify differences in adiponectin levels across a wide range of body size.

Biologic plausibility and novelty of research questions

A large number of potential correlates were selected for examination in relation to adiponectin levels including age, BMI, HDL-cholesterol, socioeconomic status (education and income), cigarette smoking, physical activity, macronutrient intakes (total calories, fat, carbohydrate, protein, as well as fiber), reproductive measures, and several co-morbidities (diabetes, heart attack or coronary artery bypass surgery, hypertension, high cholesterol, and depression). These factors were selected because reports in the literature indicated possible links to adiponectin or because they were known to be associated with body size and thus were hypothesized to be possible correlates of adiponectin as well.

Linkage studies (10, 11) as well as genome-wide association studies (8, 9) have indicated that the adiponectin locus (*ADIPOQ)* is strongly associated with adiponectin levels, supporting one of the major hypotheses of this study which was to examine polymorphisms

in this gene in relation to circulating adiponectin levels. The genes for the adiponectin receptors, *ADIPOR1* and *ADIPOR2*, were also selected because these receptors are known binding sites throughout the body for adiponectin, and it seemed plausible that variation in the receptor-encoding genes might affect their structure and thus their affinity for adiponectin leading to differences in circulating levels.

The comparison of adiponectin levels, predictors, and genetic polymorphisms in women of different race groups was an important component of this research. It has been well-established that black women are heavier than white women (12) and that black women have a higher burden of many chronic diseases (13, 14). There has been much debate about the root causes of these differences, but it seems likely that at least some of the racial disparities in incident disease and mortality may be due to differences in biologic factors. The adipokines, and adiponectin in particular, are strong candidates for exploration because they appear to play a major role in several of the metabolic pathways related to diseases such as cancer, type 2 diabetes, and CVD, and a small body of emerging evidence has indicated that there may be racial differences in protein levels in circulation. This study allowed for the largest-ever comparison of adiponectin levels and correlates in both black and white women across a wide range of age and body size.

SNP selection and Genotyping

A tag-SNP approach was employed for this study that included the selection of tag-SNPs based on both the African (YRI) and white (CEU) study samples in HapMap. The use of both data sources for tag-SNP selection ensured adequate coverage across the three genes of interest to account for the possibility that LD block sizes might be smaller among the women of African versus European descent.

The genotyping for this study was conducted as part of a larger genotyping effort, allowing the use of the Illumina GoldenGate platform, a high-throughput method with very high call rates and reproducibility.

Adjustment for population stratification

Eligibilty for this study included self-report of race/ethnicity as only "White" or "Black/African-American". However, as has been much discussed in the literature, self-reported race in such broad categories may be insufficient for dealing with the issue of population stratification which refers to substructures that may exist within race/ethnic groups that can create biased associations between genetic variants and phenotypic outcomes (15, 16). Several methodologies have been developed to adjust for population stratification and two of these were examined in this study and found to have very similar effects. Using 292 ancestry informative markers (AIM), two ancestry allelic clusters were determined using a Bayesian clustering approach implemented in the STRUCTURE software package (17). The percentage of African ancestry estimated from this method was included as a covariate in the models examining SNP-adiponectin and SNP-BMI associations. Principal components were also derived from the AIMs (18) and the first five principal components were examined as covariates. Effect estimates were essentially unchanged in models comparing the two different methods of adjustment for population stratification and thus the STRUCTURE method was selected because of the smaller degrees of freedom required in the statistical models.

Population substructure was also examined by running unadjusted linear regression models including only the black women in this sample (N=986) with log-adiponectin as the outcome variable and percentage of European ancestry (continuous) as the exposure variable. European ancestry was not significantly associated with log-adiponectin in either a crude

model (p=0.12) or in a multivariate linear regression model including age, BMI, HDL cholesterol and hypertension (p=0.36). Re-running of these models using percentage European ancestry as estimated from the entire set of 1,420 SNPs genotyped in the Komen Obesity Project resulted in nearly identical effect estimates and measures of variability. These results are somewhat in contrast with the report by Wassel Fyr et al. (19) in which the authors observed a significant association between European ancestry and log-adiponectin levels in both unadjusted models and after multivariate adjustment in 1,241 adults in the Health ABS study who self-reported their race as Black Americans. One major difference between our study and the Health ABC is the mean individual European ancestry which was 22.1% in the Health ABC study (25.1% at their Pittsburgh site and 18.7% at their Memphis site) but only 7.5% in our study (as estimated by the 276 AIM SNPs; mean European ancestry was estimated at 8.4% using the entire set of 1,420 genotyped SNPs). In this study, while broad race categories (as defined by self-reported black or white race) were clearly a major factor related to adiponectin levels, within groups of self-reported race, admixture was relatively low and not strongly associated with adiponectin levels.

These results are somewhat in contrast with the report by Wassel Fyr et al (19) in which the authors observed a significant association between European ancestry and log-adiponectin levels in both unadjusted models and after multivariate adjustment in 1,241 adults in the Health ABS study who self-reported their race as black. One major difference between our study and the Health ABC is the mean individual European ancestry which was 22.1% in the Health ABC study (25.1% at their Pittsburgh site and 18.7% at their Memphis site) but only 7.5% in our study (as estimated by the 276 AIM SNPs; mean European ancestry was estimated at 8.4% using the entire set of 1,420 genotyped SNPs). These results

in conjunction with our observation of strong differences in adiponectin levels by self-reported race groups (black v. white) indicate that while self-reported race is clearly a major factor related to adiponectin level, within groups of self-reported race, limited additional variability in racial substructure may not be strongly related to adiponectin levels.

Study Limitations

Measurement of adiponectin

The measurement of adiponectin had a coefficient of variation (CV) of 9.4%, indicating some intra-individual variation in the measurement; however, this CV is in line with CVs reported from other studies using the same assay (CV range 1.8% to 13.3%) (2, 3, 6). Variability in the adiponectin levels related to the assay itself, thus, could account for some of the effects observed in this study although it is reassuring that our results are generally in-line in both direction and magnitude with most previously published reports.

Adiponectin circulates in blood in several isoforms including a trimer, a hexamer and as a high molecular weight-form (HMW). Designed in 2005, the current study used a measurement of total adiponectin, a decision made based on the state of the science and the lack of availability of commercial assays for measurement of HMW adiponectin at the time. However, some recent work has indicated that the HMW form may be the more biologically active. In vitro, HMW adiponectin, compared to the lower order forms, was shown to have the highest binding affinity to its receptors as well as be the most potent activator of AMP kinase which plays a crucial role in the metabolic activities of adiponectin (20).

Determining which measure of adiponectin to use in epidemiologic studies includes consideration of the strength of the evidence demonstrating superiority of one isoform over the other in relation to relevant outcomes (such as glucose tolerance or chronic disease such as diabetes, cancer, or CVD) as well as the feasibility of measuring the different forms.

Some groups suggest that studies of adiponectin focus on the HMW isoform (21) while others recommend analysis of the ratio of HMW to total adiponectin or to lower order forms (22, 23). The general body of evidence evaluating HMW adiponectin is relatively small, and to date remains unclear regarding a clear preference for measurement of one isoform over another. In terms of drug efficacy, the HMW form of adiponectin appears to be the best indicator of improvements in liver insulin sensitivity after treatment with PPAR-γ agonists (24). Evidence for differences in association between total, HMW, and ratios of adiponectin forms and various disease states is less clear; in a few studies, HMW adiponectin was found to be better correlated with measure of glucose tolerance than total adiponectin (21-23). A small case-control study of Greek women reported similar odds ratios between breast cancer and total and HMW adiponectin (25). In Japanese patients, both total and HMW adiponectin, but not their ratio, were found to be positively correlated with severity of retinopathy and neuropathy in type 2 diabetics (26). HMW adiponectin but not total adiponectin was found to be associated with fruit intake in healthy Greek women (27).

As for feasibility, measurements of HMW adiponectin as recently as five years ago were labor-intensive and not suitable for large studies such as the SCCS (21). Soon after, a novel ELISA assay was developed to measure both total and HMW adiponectin (28) and this assay has very recently begun to be used in larger-scale epidemiology studies. Future work in the SCCS will likely include the measurement of multiple isoforms of adiponectin as the evidence is accumulating that these distinctions may be relevant.

A further limitation of this study related to the adiponectin measurement is the use of a single, convenience blood sample. The single blood sample provided at baseline entry into the SCCS was utilized for the measurement of adiponectin for each participant which did not

allow for the examination of inter-individual variation in adiponectin levels. Reports in the literature, however, show remarkably high correlations between adiponectin measurements across time showing that single measurements are likely sufficient for large epidemiologic studies, especially when balanced against the effort and costs that would be involved in the collection of multiple blood samples in a study as large as the SCCS (29, 30).

Additionally, SCCS participants were not required to be fasting when blood samples were taken, and thus, in this study, only 44% of the samples were collected at least 8 hours since the last meal. Most reports have showed that adiponectin does not exhibit strong variability throughout the day or in relation to meal composition (31) indicating that fasting status is not crucial in the measurement of adiponectin. In this study, analyses limited to fasting samples showed similar results to those conducted in the entire study sample.

Measurement of body size

Self-reported height and weight: Measures of height and weight used to calculate BMI were self-reported by participants during the baseline SCCS interview and represent a potential limitation of this study. Self-reported values can be unreliable and lead to biased effect estimates; indeed a recent review indicates that among women, height does tend to be over-reported while weight tends to be under-reported (32). However, in many large-scale epidemiologic studies including the SCCS during the time period of the current study, self-reported height and weight values are collected due to the higher monetary and time costs associated with collecting measured values in a consistent manner. Supporting the use of self-reported measures is a recent report using 1999-2004 National Health and Examination Survey data which showed that despite errors in self-report, BMI categories based on self-reported values still generally demonstrate good agreement with BMI categories from measured values (33). These data also showed that under-reporting was more common in

whites and among well-educated women (33) which suggests that the BMI values calculated from self-report in the SCCS may be less vulnerable to bias than in other studies of more educated, white participants. Furthermore, the self-reported height and weight data in the SCCS baseline interview are believed to be generally of high quality. First, it is expected that the in-person nature of the interview is a deterrent for gross under- or over-reporting of weight by the participants. Second, many patients have just been weighed as part of their medical visit at the Community Health Center. Finally, BMI values calculated from self-reported height and weight in the SCCS were very highly correlated with BMI values calculated from medical record data collected for approximately 25% of the participants, overall (Pearson correlation coefficient > 0.95) as well as across strata of race and BMI.

Limitations of BMI: BMI has excellent validity as a measure of absolute fat mass adjusted for height (34), but, the calculation of BMI [weight (kg)/height $(m)^2$] involves body weight which is made up of both lean body mass and fat tissue. This makes BMI a less valid measure for percent body fat than other measures that are able to account for differences in the proportion of each type of body tissue such as under-water weighing or DEXA (34).

Lack of measure of central obesity: There was no measure of central obesity (either via waist circumference or imaging techniques such as MRI/CT in this sample of women. It has been shown that the amount of centrally-deposited adipose tissue differs between black and white women at the same level of BMI (35) and further, that there are differences in the proportion of the various isoforms of adiponectin in relation to measures of central obesity across race groups (36). Given these findings, lack of measurement of central obesity is a primary limitation of this research and should be given more attention in future work.

Measurement of potential adiponectin correlates

In this study, age, BMI, HDL-cholesterol and hypertension were found to be strong predictors of adiponectin levels but other factors including socioeconomic status (education and income), cigarette smoking, physical activity, and dietary, reproductive, and co-morbidity indices were not correlated with adiponectin. Imprecise measurement of some of these factors could explain the inability to detect some associations. These factors were assessed via questionnaire including a food frequency questionnaire (FFQ) and a physical activity questionnaire (PAQ). Both the FFQ and the PAQ were designed specifically for the target study population, but, by their very nature, neither was entirely comprehensive. Many of the factors were assessed as of the time of the baseline interview and thus may not reflect past levels which may have been more influential on adiponectin levels. For physical activity, levels were low overall, which may have resulted in a lack of power to detect modest associations. Difficulty with recall by participants may have also contributed to imprecise measurement of some of these factors. Additionally, some of these factors may be more strongly correlated with HMW adiponectin which was not measured in this study.

It may also be the case that once age, BMI, HDL-cholesterol and hypertension were included in the prediction models for adiponectin, other factors (for example, diabetes and physical activity), which might have been expected to be predictive of adiponectin but are also strongly associated with age, BMI, HDL-cholesterol and hypertension, added minimal additional predictive value.

Multiple comparisons

As is common in many association studies examining multiple genes and SNPs, this study used several statistical models to assess associations between adiponectin, BMI and the genotyped SNPs. A Bonferroni correction was applied to the commonly-used type I error

rate of 0.05 to account for the multiple testing. The Bonferroni correction was selected because it is a simple, easily understood, and relatively common type of correction for these circumstances but it is not without its drawbacks. It is a conservative approach in that it is unable to account for the correlation structure inherent among a group of SNPs. A less conservative alternative might have been the use of permutation testing in order to maintain a test with appropriate type I error. However, the methodology of permutation testing has not yet been extended to allow for simultaneous adjustment for population stratification which was deemed potentially important in this study population of blacks and whites.

While the Bonferroni approach is conservative, its application did not drastically alter the interpretation of these results. There were few SNPs that were significantly associated with adiponectin or BMI at an alpha level of 0.05 (5 total for adiponectin and 9 total for BMI, out of 119 total associations examined). Further, there was no evidence of clustering of SNPs with lower p-values in any specific regions of the genes examined (Figures A.1a, A.1b, and A.1c for adiponectin and Figures A.2a, A.2b, and A2.c for BMI). None of these 14 SNPs are known to be functional variants nor have they been found in previous studies to be associated with these outcomes. They may, however, represent areas of the genes worthy of further exploration in future studies.

Future directions

These results show clear differences in adiponectin levels between black and white women after adjustment for BMI. However, while BMI is highly correlated with total body fat overall (37), there are known differences in body composition between black and white women at the same level of BMI. Several studies have shown that blacks have less visceral adipose tissue than do whites at the same BMI (35, 38-40). Thus, the measurement of visceral fat will be important in future studies attempting to determine the etiology of

differences in adiponectin levels across race groups. Sex hormones such as estrogen and androgens may also affect adiponectin expression (31) and thus their measurement may also be a useful addition to future studies examining levels and correlates of adiponectin.

With differences in adiponectin levels shown between black and white women, an exciting next step will be assessing relationships between adiponectin and incident disease in these two groups of women. In the Southern Community Cohort Study (SCCS), nested case-control studies of major cancer sites (including breast, lung, and colon) will be conducted in the near future. Measurement of adiponectin in blood samples collected at baseline entry into the SCCS (i.e. before cancer diagnosis) in these studies will be an important step in assessing the role played by adiponectin in the development of these cancers. Future studies of incident diabetes and heart disease will also be conducted shortly within the SCCS and measures of adiponectin in both the black and white participants are expected to be useful factors in untangling differences in disease rates by race. Examination of adiponectin in relation to these diseases is not limited to the SCCS, of course. The results from this study and the resulting need for future analyses highlight the importance of the continued funding of large, well-defined cohorts of individuals of diverse populations with available biospecimans for measurement of biomarkers and genetic polymorphisms.

Replication of effects observed in genetic association studies is emerging as an important research objective. In this study, SNP rs17366568 was found to be associated with adiponectin levels in white women, a finding that was recently reported by two GWAS studies of white European women. Notably, this finding was not observed in the black women in this study. Additionally, one group has found evidence to indicate that this SNP may not be functionally responsible for changes in adiponectin levels but simply in linkage

disequilibrium (LD) with an as-yet undetermined functional SNP (8). This could potentially explain why no association with adiponectin was seen in the black women in this study; if the causal allele lies elsewhere in this region, it is possible that rs17366568 did not sufficiently tag the true causal allele in the black women who, in general, would be expected to have smaller LD blocks. However, the LD patterns in our study, at least, do not support this theory as SNP rs17366568 was in a block all on its own (i.e. r^2 between SNP rs17366568 and all other genotyped SNPs was very low). Examination of this SNP, as well as sequencing in this region, in additional populations of black and white women will be important to determine whether this polymorphism or other variants nearby represent important racial differences in the genetic underpinnings of adiponectin levels.

Debate is intensifying regarding the utility of tag-SNPs (such as those used in this study) to identify important variants because this approach is unlikely to be useful for the identification of rare variants. If the common disease/rare variant (CD/RV) paradigm holds for conditions such as obesity (meaning that disease risk is due to loci with low population frequency) (31, 41), research approaches that include adequate study power for the identification of rare variants will be important in future studies. As the cost of genotyping decreases drastically with each passing year, studies in the not-too-distant future will likely consider the sequencing of entire genomes which would include both common and rare variants. The tools to meaningfully analyze large quantities of genetic data, though, are already insufficient for the current state of the science, and thus, work in this area will be successful only if the development of adequate methods is a major concurrent focus.

Conclusion

In this study, differences in adiponectin levels were observed between black and white women after adjustment for BMI. Future studies which include additional

measurements of body size and composition may help refine the magnitude of these differences. Adiponectin levels were shown to be strongly related to age, HDL-cholesterol, and hypertension but few genetic polymorphisms in three adiponectin-related genes were found with the exception of a single SNP among white women on *ADIPOQ* (rs17366568). Larger study samples or pooled studies will likely be required to detect additional variants that may have small but important cumulative effects on adiponectin levels. Observed racial differences in adiponectin levels in this study will be utilized in the design of future studies of this adipokine in racially diverse populations in relation to diseases affected by adiponectin-mediated pathways such as cancer, CVD, and type 2 diabetes. Additionally, the development of therapeutics that increase adiponectin levels for the purpose of disease prevention may be guided by the predictors of adiponectin and racial differences observed in this study.

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APPENDIX
Additional Tables and Figures

Table A.1a. Comparison of p-values for association between adiponectin and genotypes for SNPs in *ADIPOQ* by method of adjustment for population stratification

	White women		Black women	
ADIPOQ SNP[1]	**STRUCTURE[2]**	**Principal components[3]**	**STRUCTURE[2]**	**Principal components[3]**
rs864265	0.076	0.10	0.14	0.16
rs822387	0.22	0.48	0.13	0.12
rs16861194	0.73	0.91	0.50	0.51
rs182052	0.10	0.081	0.49	0.53
rs16861205	0.76	0.92	0.64	0.63
rs822391	0.47	0.46	0.85	0.92
rs16861210	0.11	0.11	0.60	0.67
rs822396	0.40	0.41	0.44	0.42
rs12495941	0.057	0.043	0.28	0.27
rs7649121	0.69	0.67	0.76	0.76
rs9877202	--	--	0.54	0.57
rs17366568	0.00036	0.00030	0.92	0.91
rs3821799	0.95	0.97	0.94	0.95
rs3774261	0.20	0.19	0.96	0.95
rs17366743	0.57	0.17	--	--
rs6444174	--	--	0.63	0.66
rs1063539	0.20	0.21	0.83	0.19
rs9842733	--	--	0.47	0.13
rs1403697	--	--	0.44	0.39
rs7641507	--	--	0.51	0.74
rs1403696	--	--	0.79	0.70
rs6444175	0.51	0.49	0.63	0.80
rs7628649	0.34	0.32	0.73	0.76
rs17373414	0.12	0.10	0.96	0.18

Table A.1b. Comparison of p-values for association between adiponectin and genotypes for SNPs in *ADIPOR1* by method of adjustment for population stratification

	White women		Black women	
ADIPOR1 SNP[1]	**STRUCTURE[2]**	**Principal components[3]**	**STRUCTURE[2]**	**Principal components[3]**
rs6672643	0.71	0.75	0.60	0.63
rs2185781	0.51	0.51	0.57	0.59
rs4336908	0.53	0.54	0.60	0.47
rs10920531	0.84	0.88	0.087	0.10
rs7539542	0.74	0.80	0.10	0.10
rs1342387	0.38	0.39	0.58	0.58
rs7518457	--	--	0.63	0.60
rs12045862	0.68	0.74	0.27	0.49
rs2275737	0.32	0.34	0.61	0.61
rs12733285	0.51	0.56	0.99	0.98
rs10753929	0.34	0.34	0.76	0.75
rs1539355	0.88	0.89	0.81	0.81
rs10800888	--	--	0.87	0.89
rs6666089	0.89	0.92	0.30	0.32
rs7523903	--	--	0.16	0.16
rs2232849	--	--	0.73	0.68
rs2232844	--	--	0.73	0.37
rs2232842	0.25	0.42	0.024	0.028

Table A.1c. Comparison of p-values for association between adiponectin and genotypes for SNPs in *ADIPOR2* by method of adjustment for population stratification

	White women		Black women	
ADIPOR2 SNP[1]	**STRUCTURE[2]**	**Principal components[3]**	**STRUCTURE[2]**	**Principal components[3]**
rs758027	--	--	0.13	0.12
rs1029629	0.60	0.61	0.57	0.60
rs7304096	--	--	0.69	0.59
rs2058033	0.47	0.49	0.069	0.13
rs7975600	0.83	0.81	0.47	0.51
rs11832817	0.31	0.31	0.16	0.17
rs12826079	0.85	0.94	--	--
rs10773982	0.72	0.71	0.45	0.43
rs11061946	0.10	0.11	0.19	0.27
rs10773983	0.0035	0.0039	0.37	0.34
rs12316367	0.054	0.071	0.72	0.68
rs10773989	0.10	0.11	0.29	0.32
rs2058112	0.17	0.17	0.94	0.94
rs12298275	--	--	0.87	0.62
rs7134070	--	--	0.60	0.56
rs7967137	0.22	0.23	0.97	0.95
rs7138701	--	--	0.77	0.80
rs11614639	0.082	0.10	0.41	0.41
rs10773991	0.067	0.083	0.76	0.73
rs4140993	--	--	0.076	0.08
rs16928751	0.22	0.23	0.96	0.96
rs2286384	0.076	0.094	0.23	0.23
rs12342	0.65	0.68	0.021	0.02
rs1044471	0.016	0.016	0.47	0.47
rs7294540	0.067	0.081	0.76	0.79
rs13219	0.071	0.089	0.45	0.46
rs2058111	0.067	0.082	0.47	0.49

Note: All models adjusted for age at baseline SCCS interview. Missing values indicate SNPs for which the minor allele frequency (MAF) was <0.01.

[1] SNPs are ordered from 5' to 3' along *ADIPOQ*

[2] STRUCTURE method included adjustment for percentage African ancestry as estimated by STRUCTURE

[3] Principal components method included adjustment for five principal components derived separately for white and black women using EIGENSTRAT

Table A.2a. Comparison of p-values for association between body mass index (BMI) and genotypes for SNPs in *ADIPOQ* by method of adjustment for population stratification

	White women		Black women	
ADIPOQ SNP[1]	**STRUCTURE[2]**	**Principal components[3]**	**STRUCTURE[2]**	**Principal components[3]**
rs864265	0.42	0.48	0.070	0.071
rs822387	0.23	0.22	0.014	0.014
rs16861194	0.75	0.95	0.86	0.86
rs182052	0.14	0.13	0.95	0.95
rs16861205	0.59	0.88	0.93	0.92
rs822391	0.073	0.08	0.21	0.23
rs16861210	0.28	0.34	0.36	0.36
rs822396	0.068	0.089	0.33	0.32
rs12495941	0.31	0.29	0.68	0.65
rs7649121	0.18	0.21	0.65	0.66
rs9877202	--	--	0.04	0.035
rs17366568	0.68	0.73	0.53	0.53
rs3821799	0.48	0.54	0.76	0.74
rs3774261	0.74	0.80	0.81	0.79
rs17366743	0.64	0.87	--	--
rs6444174	--	--	0.74	0.75
rs1063539	0.39	0.40	0.46	0.57
rs9842733	--	--	0.16	0.079
rs1403697	--	--	0.45	0.46
rs7641507	--	--	0.42	0.69
rs1403696	--	--	0.21	0.21
rs6444175	0.85	0.57	0.22	0.21
rs7628649	0.31	0.38	0.026	0.025
rs17373414	0.35	0.33	0.41	0.55

Table A.2b. Comparison of p-values for association between body mass index (BMI) and genotypes for SNPs in *ADIPOR1* by method of adjustment for population stratification

	White women		Black women	
ADIPOR1 SNP[1]	**STRUCTURE[2]**	**Principal components[3]**	**STRUCTURE[2]**	**Principal components[3]**
rs6672643	0.42	0.33	0.028	0.023
rs2185781	0.30	0.27	0.63	0.62
rs4336908	0.39	0.35	0.72	0.70
rs10920531	0.15	0.17	0.23	0.23
rs7539542	0.019	0.028	0.10	0.10
rs1342387	0.76	0.77	0.69	0.72
rs7518457		--	0.71	0.15
rs12045862	0.86	0.90	0.77	0.68
rs2275737	0.88	0.87	0.34	0.40
rs12733285	0.74	0.77	0.44	0.43
rs10753929	0.82	0.78	0.59	0.63
rs1539355	0.75	0.80	0.34	0.37
rs10800888		--	0.80	0.81
rs6666089	0.64	0.68	0.86	0.82
rs7523903		--	0.86	0.85
rs2232849		--	0.70	0.12
rs2232844		--	0.50	0.75
rs2232842	0.44	0.70	0.10	0.093

Table A.2c. Comparison of p-values for association between body mass index (BMI) and genotypes for SNPs in *ADIPOR2* by method of adjustment for population stratification

	White women		Black women	
ADIPOR2 SNP[1]	**STRUCTURE[2]**	**Principal components[3]**	**STRUCTURE[2]**	**Principal components[3]**
rs758027	--	--	0.23	0.26
rs1029629	0.55	0.25	0.96	0.95
rs7304096	--	--	0.59	0.83
rs2058033	0.51	0.55	0.77	0.96
rs7975600	0.62	0.65	0.0085	0.009
rs11832817	0.91	0.95	0.0062	0.006
rs12826079	0.42	0.47	--	--
rs10773982	0.56	0.61	0.22	0.23
rs11061946	0.68	0.76	0.87	0.98
rs10773983	0.068	0.090	0.55	0.55
rs12316367	0.27	0.33	0.03	0.038
rs10773989	0.38	0.44	0.28	0.27
rs2058112	0.20	0.21	0.79	0.77
rs12298275	--	--	0.90	0.97
rs7134070	--	--	0.96	0.96
rs7967137	0.28	0.31	0.063	0.063
rs7138701	--	--	0.92	0.89
rs11614639	0.24	0.29	0.34	0.35
rs10773991	0.20	0.25	0.21	0.22
rs4140993	--	0.60	0.55	0.52
rs16928751	0.28	0.31	0.53	0.50
rs2286384	0.20	0.25	0.11	0.12
rs12342	0.57	0.61	0.040	0.04
rs1044471	0.48	0.55	0.0040	0.0046
rs7294540	0.12	0.14	0.23	0.26
rs13219	0.15	0.18	0.15	0.16
rs2058111	0.17	0.19	0.34	0.34

Note: All models adjusted for age at baseline SCCS interview. Missing values indicate SNPs for which the minor allele frequency (MAF) was <0.01.

[1] SNPs are ordered from 5' to 3' along *ADIPOQ*

[2] STRUCTURE method included adjustment for percentage African ancestry as estimated by STRUCTURE

[3] Principal components method included adjustment for five principal components derived separately for white and black women using EIGENSTRAT

Table A.3. Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOQ* among black and white women

		Black Women		White Women	
	Genotype	N	Geometric Mean	N	Geometric Mean
rs864265	A/A	20	14.61	27	11.74
	A/C	221	12.49	261	16.04
	C/C	745	11.43	684	15.06
rs822387	A/A	446	11.96	810	15.03
	A/G	436	11.22	154	16.07
	G/G	104	12.90	8	17.95
rs16861194	A/A	566	11.88	813	15.24
	A/G	372	11.62	153	15.04
	G/G	48	10.59	6	15.58
rs182052	A/A	111	11.31	121	13.56
	A/G	474	11.56	429	15.92
	G/G	401	12.02	422	15.01
rs16861205	A/A	37	11.15	6	15.58
	A/G	321	11.50	139	15.10
	G/G	628	11.87	827	15.23
rs822391	A/A	905	11.70	607	14.85
	A/G	81	11.87	309	15.94
	G/G			56	15.30
rs16861210	A/A	28	12.61	11	19.07
	A/G	287	11.89	164	16.68
	G/G	671	11.61	797	14.88
rs822396	A/A	619	11.77	628	14.85
	A/G	320	11.43	296	16.05
	G/G	47	13.09	48	14.91
rs12495941	A/A	143	12.14	104	14.00
	A/C	451	11.23	448	15.95
	C/C	392	12.15	420	14.76
rs7649121	A/A	779	11.72	663	15.38
	A/T	196	11.65	287	14.94
	T/T	11	13.09	22	13.75

Table A.3. (continued) Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOQ* among black and white women

		Black Women		White Women	
	Genotype	N	Geometric Mean	N	Geometric Mean
rs9877202	A/A	701	11.70		
	A/G	252	12.00		
	G/G	33	10.12		
rs17366568	A/A	1	7.64	19	9.22
	A/G	28	11.82	225	13.63
	G/G	957	11.72	728	15.95
rs3821799	A/A	324	11.82	194	15.08
	A/G	486	11.63	456	15.00
	G/G	176	11.77	322	15.60
rs3774261	A/A	309	11.62	145	16.06
	A/G	495	11.79	431	15.47
	G/G	182	11.69	396	14.64
rs17366743	A/A			921	15.20
	A/G			48	16.22
	G/G			3	7.42
rs6444174	A/A	691	11.85		
	A/G	262	11.30		
	G/G	33	12.43		
rs1063539	C/C	583	11.62	693	14.93
	C/G	362	11.74	215	16.21
	G/G	2	28.83	15	15.79
rs9842733	A/A	8	19.09		
	A/T	161	11.11		
	T/T	817	11.79		
rs1403697	A/A	756	11.71		
	A/G	215	11.54		
	G/G	15	15.03		
rs7641507	A/A	5	10.69		
	A/G	144	11.28		
	G/G	837	11.80		

Table A.3. (continued) Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOQ* among black and white women

		Black Women		White Women	
	Genotype	N	Geometric Mean		Genotype
rs1403696	A/A	45	12.72		
	A/G	326	11.44		
	G/G	615	11.80		
rs6444175	A/A	76	12.74	75	16.02
	A/G	454	11.57	352	15.39
	G/G	456	11.71	545	14.99
rs7628649	A/A	105	12.13	14	16.53
	A/G	452	11.48	186	16.07
	G/G	429	11.87	772	14.99
rs17373414	A/A	1	46.30	15	21.42
	A/G	20	11.01	199	14.77
	G/G	965	11.72	758	15.23

Table A.4. Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOR1* among black and white women

		Black women		White women	
SNP	Genotype	N	Geometric Mean	N	Geometric Mean
rs6672643	A/A	546	11.56	732	15.25
	A/G	366	11.84	213	15.36
	G/G	73	12.39	26	13.09
rs2185781	A/A	41	12.13	43	15.04
	A/G	291	12.11	295	14.62
	G/G	654	11.52	634	15.51
rs4336908	A/A	8	15.29	43	15.04
	A/G	129	11.23	293	14.65
	G/G	849	11.77	636	15.49
rs10920531	A/A	297	11.82	146	15.20
	A/C	456	12.16	409	15.00
	C/C	233	10.79	417	15.43
rs7539542	C/C	394	11.87	101	15.93
	C/G	435	12.02	393	15.18
	G/G	157	10.57	478	15.09
rs1342387	A/A	256	12.08	219	14.62
	A/G	479	11.72	441	15.84
	G/G	250	11.36	312	14.77
rs7518457	A/A	873	11.68		
	A/G	108	12.17		
	G/G	5	8.69		
rs12045862	A/A	9	10.70	72	15.75
	A/G	159	11.20	366	15.57
	G/G	818	11.83	534	14.90
rs2275737	A/A	195	12.18	206	14.36
	A/C	484	11.41	424	15.80
	C/C	307	11.92	342	15.02
rs12733285	A/A	50	12.18	91	14.18
	A/G	321	11.70	400	15.50
	G/G	615	11.69	481	15.17

Table A.4. (continued) Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in ADIPOR1 among black and white women

		Black women		White women	
SNP	Genotype	N	Geometric Mean	N	Geometric Mean
rs10753929	A/A	41	12.17	21	18.62
	A/G	304	11.86	207	14.56
	G/G	641	11.62	744	15.31
rs1539355	A/A	290	11.53	475	15.36
	A/G	491	11.67	397	15.06
	G/G	205	12.11	100	15.09
rs10800888	A/A	12	12.31		
	A/G	224	11.79		
	G/G	750	11.69		
rs6666089	A/A	19	11.14	96	14.94
	A/G	229	11.05	395	15.13
	G/G	738	11.95	481	15.33
rs7523903	C/C	13	12.12		
	C/G	391	12.38		
	G/G	582	11.28		
rs2232849	A/A	9	9.62		
	A/G	162	12.03		
	G/G	815	11.68		
rs2232844	A/A	816	11.68		
	A/G	161	12.10		
	G/G	9	8.95		
rs2232842	A/A	694	11.39	917	15.10
	A/G	269	12.23	53	16.88
	G/G	23	16.68	2	32.82

Table A.5 Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOR2* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Geometric mean**	**N**	**Geometric mean**
rs758027	A/A	800	11.95		
	A/G	174	10.97		
	G/G	11	8.34		
rs1029629	A/A	553	11.43	489	14.99
	A/C	383	12.21	391	15.20
	C/C	50	11.25	91	16.51
rs7304096	A/A	913	11.74		
	A/G	72	11.52		
	G/G	1	5.82		
rs2058033	A/A	935	11.59	743	14.99
	A/C	50	14.50	206	15.70
	C/C	1	6.45	23	18.49
rs7975600	A/A	11	10.13	21	16.04
	A/T	230	12.36	237	15.22
	T/T	745	11.55	714	15.19
rs11832817	A/A	15	10.15	79	17.24
	A/G	278	12.67	382	15.31
	G/G	693	11.39	511	14.85
rs12826079	A/A			6	14.27
	A/G			115	15.44
	G/G			851	15.19
rs10773982	A/A	387	11.43	451	14.97
	A/G	465	12.10	413	15.22
	G/G	134	11.28	108	16.23
rs11061946	A/A	1	6.45	4	29.74
	A/G	21	15.51	126	16.86
	G/G	964	11.65	841	14.91
rs10773983	A/A	547	11.60	105	16.03
	A/G	374	11.63	391	16.69
	G/G	65	13.36	475	13.90

Table A.5 (continued) Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOR2* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Geometric mean**	**N**	**Geometric mean**
rs12316367	A/A	20	10.69	279	13.88
	A/G	257	11.96	480	15.44
	G/G	709	11.66	213	16.59
rs10773989	A/A	529	11.55	228	16.82
	A/G	395	12.19	502	14.83
	G/G	62	10.37	242	14.60
rs2058112	A/A	46	11.76	12	20.49
	A/G	330	11.53	232	16.19
	G/G	610	11.82	728	14.84
rs12298275	A/A	908	11.72		
	A/G	77	11.83		
	G/G	1	5.82		
rs7134070	A/A	729	11.89		
	A/G	238	11.24		
	G/G	19	11.16		
rs7967137	A/A	498	11.80	728	14.84
	A/G	403	11.61	231	16.22
	G/G	85	11.76	13	19.35
rs7138701	A/A	38	10.45		
	A/G	301	11.76		
	G/G	647	11.78		
rs11614639	A/A	296	11.15	309	14.06
	A/C	504	11.81	473	15.47
	C/C	186	12.42	190	16.58
rs10773991	A/A	34	10.97	279	13.98
	A/G	322	11.97	476	15.33
	G/G	630	11.63	217	16.66
rs4140993	A/A	691	11.50		
	A/C	266	12.58		
	C/C	29	9.57		

Table A.5 (continued) Counts and unadjusted geometric means for adiponectin levels (ug/ml) by genotypes of SNPs in *ADIPOR2* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Geometric mean**	**N**	**Geometric mean**
rs16928751	A/A	48	11.88	13	19.35
	A/G	341	11.57	231	16.22
	G/G	597	11.79	728	14.84
rs2286384	C/C	82	10.47	280	14.02
	C/G	451	11.57	475	15.31
	G/G	453	12.11	217	16.66
rs12342	A/A	29	12.17	101	16.21
	A/G	313	12.99	392	15.37
	G/G	644	11.13	479	14.88
rs1044471	A/A	37	10.20	225	13.40
	A/G	327	11.91	497	15.48
	G/G	622	11.72	250	16.47
rs7294540	A/A	724	11.72	173	16.42
	A/C	247	11.79	471	15.71
	C/C	15	10.38	328	13.95
rs13219	A/A	22	10.07	327	14.06
	A/G	268	12.12	464	15.47
	G/G	696	11.62	181	16.81
rs2058111	A/A	640	11.64	180	16.84
	A/C	314	12.02	461	15.47
	C/C	30	10.24	329	14.07

Table A.6 Counts and unadjusted means for body mass index (kg/m2) by genotypes of SNPs in *ADIPOQ* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs864265	A/A	20	32.05	27	31.79
	A/C	223	31.09	263	30.54
	C/C	747	30.17	687	30.21
rs822387	A/A	448	30.32	815	30.23
	A/G	438	30.87	154	31.05
	G/G	104	28.89	8	28.18
rs16861194	A/A	567	30.41	817	30.31
	A/G	375	30.49	154	30.50
	G/G	48	29.93	6	30.74
rs182052	A/A	111	30.54	121	30.52
	A/G	477	30.32	431	29.88
	G/G	402	30.49	425	30.76
rs16861205	A/A	37	30.38	6	30.74
	A/G	324	30.54	140	30.62
	G/G	629	30.35	831	30.29
rs822391	A/A	909	30.36	610	30.47
	A/G	81	30.97	311	29.81
	G/G			56	31.91
rs16861210	A/A	28	31.50	11	31.13
	A/G	290	30.79	164	31.05
	G/G	672	30.21	802	30.19
rs822396	A/A	622	30.64	631	30.45
	A/G	321	30.12	298	29.83
	G/G	47	29.47	48	32.14
rs12495941	A/A	143	30.00	104	30.93
	A/C	453	30.47	452	30.01
	C/C	394	30.50	421	30.56
rs7649121	A/A	783	30.35	667	30.57
	A/T	196	30.58	288	29.96
	T/T	11	31.74	22	28.52

Table A.6 (continued) Counts and unadjusted means for body mass index (kg/m^2) by genotypes of SNPs in *ADIPOQ* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs9877202	A/A	704	30.71		
	A/G	253	29.59		
	G/G	33	30.39		
rs17366568	A/A	1	34.70	19	31.31
	A/G	28	29.26	226	30.09
	G/G	961	30.44	732	30.39
rs3821799	A/A	324	30.37	195	30.65
	A/G	489	30.34	460	30.44
	G/G	177	30.70	322	30.01
rs3774261	A/A	309	30.47	146	30.26
	A/G	498	30.31	435	30.51
	G/G	183	30.61	396	30.18
rs17366743	A/A			926	30.37
	A/G			48	29.97
	G/G			3	28.68
rs6444174	A/A	693	30.51		
	A/G	264	30.25		
	G/G	33	29.79		
rs1063539	C/C	585	30.59	697	30.15
	C/G	364	30.28	216	30.82
	G/G	2	26.84	15	30.61
rs9842733	A/A	8	25.66		
	A/T	162	29.98		
	T/T	820	30.55		
rs1403697	A/A	758	30.55		
	A/G	217	29.94		
	G/G	15	30.39		
rs7641507	A/A	5	31.67		
	A/G	144	30.82		
	G/G	841	30.34		

Table A.6 (continued) Counts and unadjusted means for body mass index (kg/m^2) by genotypes of SNPs in *ADIPOQ* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs1403696	A/A	46	30.03		
	A/G	328	29.98		
	G/G	616	30.68		
rs6444175	A/A	76	30.58	75	30.25
	A/G	454	30.81	356	30.19
	G/G	460	30.00	546	30.46
rs7628649	A/A	106	29.61	14	29.43
	A/G	454	30.03	187	30.97
	G/G	430	31.02	776	30.21
rs17373414	A/A	1	24.14	15	27.91
	A/G	20	29.37	199	30.49
	G/G	969	30.44	763	30.35

Table A.7. Counts and unadjusted means for body mass index (kg/m2) by genotypes of SNPs in *ADIPOR1* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs6672643	A/A	550	29.94	735	30.42
	A/G	366	30.84	215	29.90
	G/G	73	31.80	26	31.38
rs2185781	A/A	41	30.81	43	29.86
	A/G	293	30.15	297	30.84
	G/G	656	30.51	637	30.14
rs4336908	A/A	8	31.62	43	29.86
	A/G	130	30.09	295	30.78
	G/G	852	30.45	639	30.17
rs10920531	A/A	297	30.71	148	29.77
	A/C	458	30.55	409	30.82
	C/C	235	29.78	420	30.08
rs7539542	C/C	395	30.78	102	28.87
	C/G	436	30.45	394	30.89
	G/G	159	29.42	481	30.21
rs1342387	A/A	257	30.40	221	30.24
	A/G	480	30.57	443	30.23
	G/G	252	30.08	313	30.57
rs7518457	A/A	876	30.38		
	A/G	109	30.43		
	G/G	5	36.13		
rs12045862	A/A	9	31.62	72	29.93
	A/G	160	30.17	368	30.41
	G/G	821	30.45	537	30.35
rs2275737	A/A	196	30.08	208	30.20
	A/C	485	30.74	426	30.30
	C/C	309	30.11	343	30.48
rs12733285	A/A	51	30.52	91	30.64
	A/G	322	30.77	403	30.16
	G/G	617	30.22	483	30.44

Table A.7. (continued) Counts and unadjusted means for body mass index (kg/m^2) by genotypes of SNPs in *ADIPOR1* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs10753929	A/A	41	29.51	21	29.58
	A/G	304	30.41	210	30.21
	G/G	645	30.47	746	30.40
rs1539355	A/A	292	30.24	478	30.19
	A/G	492	30.71	399	30.43
	G/G	206	29.97	100	30.69
rs10800888	A/A	12	30.65		
	A/G	224	30.69		
	G/G	754	30.33		
rs6666089	A/A	19	30.79	96	30.95
	A/G	231	30.21	397	30.30
	G/G	740	30.47	484	30.26
rs7523903	C/C	13	30.46		
	C/G	392	30.28		
	G/G	585	30.50		
rs2232849	A/A	9	34.65		
	A/G	163	30.04		
	G/G	818	30.44		
rs2232844	A/A	820	30.35		
	A/G	161	30.77		
	G/G	9	29.99		
rs2232842	A/A	698	30.51	922	30.38
	A/G	269	30.41	53	29.78
	G/G	23	27.68	2	27.52

Table A.8. Counts and unadjusted means for body mass index (kg/m2) by genotypes of SNPs in *ADIPOR2* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs758027	A/A	804	30.24		
	A/G	174	31.20		
	G/G	11	30.99		
rs1029629	A/A	555	30.43	490	30.58
	A/C	385	30.35	394	30.11
	C/C	50	30.68	92	30.18
rs7304096	A/A	917	30.38		
	A/G	72	30.82		
	G/G	1	30.90		
rs2058033	A/A	939	30.41	744	30.47
	A/C	50	30.58	209	29.88
	C/C	1	30.18	24	30.29
rs7975600	A/A	11	33.11	21	29.58
	A/T	232	29.36	238	30.67
	T/T	747	30.70	718	30.26
rs11832817	A/A	15	33.19	80	30.24
	A/G	280	29.46	386	30.25
	G/G	695	30.74	511	30.43
rs12826079	A/A			6	27.59
	A/G			117	30.01
	G/G			854	30.41
rs10773982	A/A	389	30.57	451	30.57
	A/G	466	30.10	417	30.09
	G/G	135	31.08	109	30.38
rs11061946	A/A	1	30.18	4	31.86
	A/G	21	29.99	129	30.10
	G/G	968	30.42	843	30.38
rs10773983	A/A	550	30.61	106	29.56
	A/G	375	30.10	395	29.99
	G/G	65	30.58	475	30.82

Table A.8. (continued) Counts and unadjusted means for body mass index (kg/m^2) by genotypes of SNPs in *ADIPOR2* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs12316367	A/A	20	33.80	279	30.78
	A/G	257	30.11	483	30.31
	G/G	713	30.43	215	29.84
rs10773989	A/A	533	30.27	230	29.92
	A/G	395	30.43	505	30.34
	G/G	62	31.56	242	30.76
rs2058112	A/A	46	30.92	12	27.20
	A/G	331	30.30	233	30.13
	G/G	613	30.44	732	30.46
rs12298275	A/A	912	30.41		
	A/G	77	30.50		
	G/G	1	30.90		
rs7134070	A/A	731	30.41		
	A/G	239	30.44		
	G/G	20	30.20		
rs7967137	A/A	500	30.61	732	30.46
	A/G	405	29.94	232	30.11
	G/G	85	31.52	13	27.80
rs7138701	A/A	39	30.65		
	A/G	302	30.30		
	G/G	649	30.45		
rs11614639	A/A	297	30.81	309	30.65
	A/C	505	30.37	476	30.42
	C/C	188	29.90	192	29.67
rs10773991	A/A	34	32.24	279	30.77
	A/G	322	30.19	479	30.37
	G/G	634	30.43	219	29.73
rs4140993	A/A	692	30.51		
	A/C	269	30.06		
	C/C	29	31.30		

Table A.8. (continued) Counts and unadjusted means for body mass index (kg/m^2) by genotypes of SNPs in *ADIPOR2* among black and white women

		Black women		White women	
SNP	**Genotype**	**N**	**Mean**	**N**	**Mean**
rs16928751	A/A	48	31.10	13	27.80
	A/G	343	30.17	232	30.11
	G/G	599	30.50	732	30.46
rs2286384	C/C	82	31.29	280	30.78
	C/G	451	30.68	478	30.37
	G/G	457	30.00	219	29.73
rs12342	A/A	29	29.03	102	30.34
	A/G	315	29.72	396	30.08
	G/G	646	30.81	479	30.56
rs1044471	A/A	37	33.61	225	30.57
	A/G	327	30.52	500	30.44
	G/G	626	30.17	252	29.93
rs7294540	A/A	728	30.44	175	29.54
	A/C	247	30.19	474	30.33
	C/C	15	32.87	328	30.79
rs13219	A/A	22	32.80	327	30.73
	A/G	268	30.15	467	30.37
	G/G	700	30.44	183	29.58
rs2058111	A/A	644	30.39	182	29.61
	A/C	314	30.31	464	30.36
	C/C	30	31.98	329	30.73

Table A.9. Step-by-step confounding assessment for creation of a linear regression model using backwards model selection with log-adiponectin as outcome and BMI as primary exposure among white women.

Covariate	BMI Change-in-estimate (%)	Model action
BMI	--	Forced
Age at interview	--	Forced
Sample selection (pilot/Komen)	--	Forced
Current PA recommendation met (Yes/No)	0.02	Dropped (step 1)
Menopause (Pre/Post)	0.01	Dropped (step 2)
Education	0.01	Dropped (step 3)
Total protein intake	0.02	Dropped (step 4)
Number of live births	0.1	Dropped (step 5)
Alcohol consumption	0.04	Dropped (step 6)
Total energy intake	0.09	Dropped (step 7)
Depression (Yes/No)	0.1	Dropped (step 8)
Total physical activity	0.02	Dropped (step 9)
Age at first menstrual period	0.03	Dropped (step 10)
Diabetes	0.3	Dropped (step 11)
Total fat intake	0.06	Dropped (step 12)
Cigarette smoking status	0.3	Dropped (step 13)
High cholesterol (Yes/No)	0.2	Dropped (step 14)
Total carbohydrate intake	0.7	Dropped (step 15)
Income	1.5	Dropped (step 16)
Heart disease (Yes/No)	2.6	Dropped (step 17)
Hypertension (Yes/No)	1.4	Dropped (step 18)
HDL cholesterol	37.0	Retained

Table A.10. Step-by-step confounding assessment for creation of a linear regression model using backwards model selection with log-adiponectin as outcome and BMI as primary exposure among black women.

Covariate	BMI Change-in-estimate (%)	Model action
BMI	--	Forced
Age at interview	--	Forced
Sample selection (pilot/Komen)	--	Forced
Total energy intake	0.04	Dropped (step 1)
Menopause (Pre/Post)	0.04	Dropped (step 2)
Depression (Yes/No)	0.04	Dropped (step 3)
Total fat intake	0.01	Dropped (step 4)
High cholesterol (Yes/No)	0.1	Dropped (step 5)
Total physical activity	0.01	Dropped (step 6)
Current PA recommendation met (Yes/No)	0.004	Dropped (step 7)
Total protein intake	0.4	Dropped (step 8)
Heart disease (Yes/No)	0.3	Dropped (step 9)
Education	0.7	Dropped (step 10)
Total carbohydrate intake	0.08	Dropped (step 11)
Number of live births	1.7	Dropped (step 12)
Income	0.1	Dropped (step 13)
Diabetes (Yes/No)	2.9	Dropped (step 14)
Hypertension (Yes/No)	4.8	Dropped (step 15)
Age at first menstrual period	1.3	Dropped (step 16)
Alcohol consumption	2.9	Dropped (step 17)
Cigarette smoking status	3.5	Dropped (step 18)
HDL cholesterol	56.1	Retained

Table A.11. Step-by-step modeling decisions for 992 white women using Akaike Information Criteria (AIC) for multiple linear regression model fit evaluation of prediction model for log-adiponectin as outcome.

Model	Model covariates in model with lowest AIC	AIC (Δ AIC)
1	Sample selection BMI	-694.0
2	Sample selection BMI HDL cholesterol	-812.1 (118.1)
3	Sample selection BMI HDL cholesterol Age	-835.1 (23.0)
4	Sample selection BMI HDL cholesterol Age Hypertension	-836.2 (1.1)
STOP: No other covariates change AIC > 1 unit		

Δ AIC = change in AIC from previous model

Sample selection = Participant selected from SCCS Biospeciman pilot versus Komen Obesity Project

Table A.12. Step-by-step modeling decisions for 992 black women using Akaike Information Criteria (AIC) for multiple linear regression model fit evaluation of prediction model for log-adiponectin as outcome.

Model	Model covariates in model with lowest AIC	AIC (Δ AIC)
1	Sample selection BMI	-629.2
2	Sample selection BMI HDL cholesterol	-730.6 (101.3)
3	Sample selection BMI HDL cholesterol Age	-740.7 (10.8)
4	Sample selection BMI HDL cholesterol Age Hypertension	-743.2 (2.5)
STOP: No other covariates change AIC > 1 unit		

Δ AIC = change in AIC from previous model

Sample selection = Participant selected from SCCS Biospeciman pilot versus Komen Obesity Project

Table A.13. Beta coefficients and standard errors for body mass index (BMI) term in race-stratified linear regression models with log-adiponectin as the outcome and adjustment for HDL cholesterol and hypertension, by categories of year of birth.

	Black women		**White women**	
Year of birth	N	Beta (se)	N	Beta (se)
1924 – 1939	100	-0.031 (0.014)	72	-0.016 (0.011)
1940 – 1949	166	-0.015 (0.008)	204	-0.012 (0.009)
1950 – 1959	419	-0.034 (0.005)	389	-0.018 (0.006)
1960 – 1966	310	-0.028 (0.006)	331	-0.017 (0.006)

Table A.14. Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOQ* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs864265	A/A	14.44	2	0.14	11.38	2	0.076
	A/C	12.43			15.88		
	C/C	11.45			15.14		
rs822387	A/A	11.87	2	0.13	15.02	1	0.22
	A/G	11.25			16.23		
	G/G	13.12					
rs16861194	A/A	11.91	2	0.50	15.27	1	0.73
	A/G	11.60			14.93		
	G/G	10.54					
rs182052	A/A	11.42	2	0.49	13.54	2	0.10
	A/G	11.46			15.87		
	G/G	12.11			15.06		
rs16861205	A/A	11.15	2	0.64	14.95	1	0.76
	A/G	11.42					
	G/G	11.91			15.26		
rs822391	A/A	11.73	1	0.85	14.99	2	0.47
	A/G	11.54			15.83		
	G/G				14.34		
rs16861210	A/A	12.94	2	0.60	17.64	2	0.11
	A/G	11.97			16.84		
	G/G	11.56			14.87		
rs822396	A/A	11.80	2	0.44	14.99		
	A/G	11.38			15.89		
	G/G	13.06			14.04	2	0.40
rs12495941	A/A	12.14	2	0.28	13.99	2	0.057
	A/C	11.26			16.14		
	C/C	12.12			14.58		

Table A.14. (continued) Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOQ* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs7649121	A/A	11.74	2	0.76	15.36	2	0.69
	A/T	11.52			14.99		
	T/T	13.52			13.58		
rs9877202	A/A	11.69	2	0.54			
	A/G	11.99					
	G/G	10.35					
rs17366568	A/A	11.55	1	0.92	9.30	2	0.00036
	A/G				13.72		
	G/G	11.72			15.91		
rs3821799	A/A	11.85	2	0.94	15.21	2	0.95
	A/G	11.64			15.11		
	G/G	11.70			15.36		
rs3774261	A/A	11.65	2	0.96	16.21	2	0.20
	A/G	11.80			15.57		
	G/G	11.61			14.49		
rs17366743	A/A				15.16	1	0.57
	A/G				16.10		
rs6444174	A/A	11.86	2	0.63			
	A/G	11.30					
	G/G	12.19					
rs1063539	C/C	11.64	1	0.83	14.87	2	0.20
	C/G	11.76			16.49		
	G/G				15.06		
rs9842733	A/A	11.29	1	0.47			
	T/T	11.81					
rs1403697	A/A	11.73	2	0.44			
	A/G	11.48					
	G/G	14.72					

Table A.14. (continued) Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOQ* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs7641507	A/A	11.31	1	0.51			
	G/G	11.79					
rs1403696	A/A	12.29	2	0.79			
	A/G	11.50					
	G/G	11.80					
rs6444175	A/A	12.64	2	0.63	15.85	2	0.51
	A/G	11.68			15.65		
	G/G	11.61			14.85		
rs7628649	A/A	12.00	2	0.73	16.01	2	0.34
	A/G	11.49			16.30		
	G/G	11.89			14.95		
rs17373414	A/A	11.81	1	0.96	22.25	2	0.12
	A/G				14.82		
	G/G	11.72			15.20		

Note: Models include adjustment for age at baseline SCCS interview (continuous) and percentage African ancestry (continuous)

Table A.15. Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOR1* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs6672643	G/G	12.50	2	0.60	13.52	2	0.71
	A/A	11.51			15.24		
	A/G	11.89			15.33		
rs2185781	A/A	11.97	2	0.57	15.67	2	0.51
	A/G	12.14			14.60		
	G/G	11.52			15.48		
rs4336908	A/A	11.37	1	0.60	15.67	2	0.53
	A/G				14.62		
	G/G	11.78			15.46		
rs10920531	A/A	11.83	2	0.087	15.37	2	0.84
	A/C	12.19			14.96		
	C/C	10.73			15.40		
rs7539542	C/C	11.90	2	0.10	16.05	2	0.74
	C/G	12.04			15.11		
	G/G	10.46			15.12		
rs1342387	A/A	12.06	2	0.58	14.70	2	0.38
	A/G	11.77			15.77		
	G/G	11.28			14.81		
rs7518457	A/A	11.67	1	0.63			
	A/G	12.09					
rs12045862	A/A	11.08	1	0.27	15.87	2	0.68
	A/G				15.48		
	G/G	11.85			14.94		
rs2275737	A/A	12.18	2	0.61	14.44	2	0.32
	A/C	11.48			15.79		
	C/C	11.81			14.98		

Table A.15. (continued) Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOR1* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs12733285	A/A	11.92	2	0.99	14.05	2	0.51
	A/G	11.71			15.51		
	G/G	11.70			15.20		
rs10753929	A/A	12.31	2	0.76	18.82	2	0.34
	A/G	11.94			14.76		
	G/G	11.58			15.25		
rs1539355	G/G	12.05	2	0.81	15.11	2	0.88
	A/A	11.55			15.40		
	A/G	11.68			15.02		
rs10800888	A/A	12.86	2	0.87			
	A/G	11.84					
	G/G	11.67					
rs6666089	A/A	10.73	2	0.30	14.90	2	0.89
	A/G	11.05			15.09		
	G/G	11.96			15.38		
rs7523903	C/C	12.15	2	0.16			
	C/G	12.36					
	G/G	11.30					
rs2232849	A/A	11.92	1	0.73			
	G/G	11.68					
rs2232844	A/A	11.68	1	0.73			
	A/G	11.92					
rs2232842	A/G	12.13	2	0.024	16.99	1	0.25
	A/A	11.42			15.11		
	G/G	16.92					

Note: Models include adjustment for age at baseline SCCS interview (continuous) and percentage African ancestry (continuous)

Table A.16. Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOR2* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs758027	G/G	8.46	2	0.13			
	A/G	11.02					
	A/A	11.94					
rs1029629	C/C	11.13	2	0.57	16.32	2	0.60
	A/C	12.06			15.24		
	A/A	11.54			15.00		
rs7304096	A/A	11.75	1	0.69			
	A/G	11.34					
rs2058033	A/A	11.60	1	0.069	15.04	2	0.47
	A/C	14.02			15.58		
	C/C				17.86		
rs7975600	A/A	9.79	2	0.47	16.73	2	0.83
	A/T	12.20			15.19		
	T/T	11.60			15.17		
rs11832817	A/A	9.87	2	0.16	17.02	2	0.31
	A/G	12.50			15.30		
	G/G	11.46			14.88		
rs12826079	A/A				15.39	1	0.85
	G/G				15.19		
rs10773982	G/G	11.08	2	0.45	16.00	2	0.72
	A/G	12.05			15.23		
	A/A	11.56			15.01		
rs11061946	A/A	14.30	1	0.19	16.76	1	0.10
	G/G	11.67			14.97		
rs10773983	G/G	13.25	2	0.37	14.05	2	0.0035
	A/G	11.57			16.60		
	A/A	11.65			15.61		

Table A.16. (continued) Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOR2* SNPs.

		Black women			White women		
SNP	**Genotype**	**Adiponectin geometric mean**	**df**	**p-value**	**Adiponectin geometric mean**	**df**	**p-value**
rs12316367	A/A	10.58	2	0.72	13.99	2	0.054
	A/G	11.97			15.49		
	G/G	11.66			16.30		
rs10773989	G/G	10.49	2	0.29	14.63	2	0.10
	A/G	12.13			14.88		
	A/A	11.57			16.65		
rs2058112	A/A	11.82	2	0.94	19.68	2	0.17
	A/G	11.58			16.09		
	G/G	11.78			14.88		
rs12298275	A/A	11.73	1	0.87			
	A/G	11.57					
rs7134070	G/G	11.55	2	0.60			
	A/G	11.25					
	A/A	11.88					
rs7967137	G/G	11.72	2	0.97	18.42	2	0.22
	A/G	11.64			16.13		
	A/A	11.78			14.88		
rs7138701	A/A	10.80	2	0.77			
	A/G	11.83					
	G/G	11.72					
rs11614639	C/C	12.35	2	0.41	16.25	2	0.082
	A/C	11.75			15.54		
	A/A	11.29			14.14		
rs10773991	A/A	11.15	2	0.76	14.09	2	0.067
	A/G	11.98			15.37		
	G/G	11.62			16.40		
rs4140993	C/C	9.83	2	0.076			
	A/C	12.63					
	A/A	11.47					

Table A.16. (continued) Adjusted geometric means for adiponectin levels (ug/ml) and p-values from race-stratified linear regression models examining associations between adiponectin and *ADIPOR2* SNPs.

		Black women			White women		
SNP	Genotype	Adiponectin geometric mean	df	p-value	Adiponectin geometric mean	df	p-value
rs16928751	A/A	11.92	2	0.96	18.42	2	0.22
	A/G	11.62			16.13		
	G/G	11.76			14.88		
rs2286384	C/C	10.51	2	0.23	14.14	2	0.076
	C/G	11.57			15.35		
	G/G	12.10			16.40		
rs12342	A/A	12.15	2	0.021	15.99	2	0.65
	A/G	12.85			15.37		
	G/G	11.19			14.93		
rs1044471	A/A	10.21	2	0.47	13.50	2	0.016
	A/G	11.92			15.56		
	G/G	11.71			16.20		
rs7294540	C/C	10.23	2	0.76	14.10	2	0.067
	A/C	11.76			15.73		
	A/A	11.74			16.04		
rs13219	A/A	10.13	2	0.45	14.18	2	0.071
	A/G	12.13			15.51		
	G/G	11.62			16.42		
rs2058111	C/C	10.27	2	0.47	14.19	2	0.067
	A/C	12.05			15.51		
	A/A	11.63			16.48		

Note: Models include adjustment for age at baseline SCCS interview (continuous) and percentage African ancestry (continuous)

Table A.17. Adjusted means for body mass index (BMI, kg/m2) and p-values from race stratified linear regression models examining BMI and ADIPOQ SNPs.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs864265	A/A	32.10	2	0.070	31.77	2	0.42
	A/C	31.12			30.53		
	C/C	30.16			30.21		
rs822387	A/A	30.38	2	0.014	30.23	1	0.23
	A/G	30.83			30.91		
	G/G	28.81					
rs16861194	A/A	30.42	2	0.86	30.31		
	A/G	30.46			30.50	1	0.75
	G/G	29.93					
rs182052	A/A	30.52	2	0.95	30.52	2	0.14
	A/G	30.35			29.87		
	G/G	30.46			30.77		
rs16861205	A/A	30.32	2	0.93	30.61	1	0.59
	A/G	30.52					
	G/G	30.36			30.29		
rs822391	A/A	30.34	1	0.21	30.48	2	0.073
	A/G	31.27			29.80		
	G/G				31.86		
rs16861210	A/A	31.33	2	0.36	31.07	2	0.28
	A/G	30.76			31.06		
	G/G	30.23			30.18		
rs822396	A/A	30.63	2	0.33	30.45	2	0.068
	A/G	30.13			29.82		
	G/G	29.54			32.10		
rs12495941	A/A	29.98	2	0.68	30.94	2	0.31
	A/C	30.46			30.01		
	C/C	30.51			30.55		
rs7649121	A/A	30.34	2	0.65	30.57	2	0.18
	A/T	30.66			29.96		
	T/T	31.71			28.50		

Table A.17. (continued) Adjusted means for body mass index (BMI, kg/m^2) and p-values from race stratified linear regression models examining BMI and *ADIPOQ* SNPs.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs9877202	A/A	30.74	2	0.037			
	A/G	29.54					
	G/G	30.28					
rs17366568	A/A	29.67	1	0.53	31.32	2	0.68
	A/G				30.10		
	G/G	30.44			30.39		
rs3821799	A/A	30.34	2	0.76	30.66	2	0.48
	A/G	30.35			30.45		
	G/G	30.74			30.00		
rs3774261	A/A	30.43	2	0.81	30.27	2	0.74
	A/G	30.31			30.52		
	G/G	30.66			30.17		
rs17366743	A/A				30.37	1	0.64
	A/G				29.92		
rs6444174	A/A	30.51	2	0.74			
	A/G	30.24					
	G/G	29.86					
rs1063539	C/C	30.59	1	0.46	30.14	2	0.39
	C/G	30.27			30.84		
	G/G				30.56		
rs9842733	A/A	29.80	1	0.16			
	T/T	30.54					
rs1403697	A/A	30.55	2	0.45			
	A/G	29.93					
	G/G	30.65					
rs7641507	A/A	30.80	1	0.42			
	G/G	30.35					
rs1403696	A/A	30.18	2	0.21			
	A/G	29.93					
	G/G	30.69					

Table A.17. (continued) Adjusted means for body mass index (BMI, kg/m^2) and p-values from race stratified linear regression models examining BMI and *ADIPOQ* SNPs.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs6444175	A/A	30.55	2	0.22	30.24	2	0.85
	A/G	30.77			30.20		
	G/G	30.04			30.45		
rs7628649	A/A	29.62	2	0.026	29.40	2	0.31
	A/G	30.03			30.98		
	G/G	31.02			30.20		
rs17373414	A/A	29.28	1	0.41	27.94	2	0.35
	A/G				30.50		
	G/G	30.44			30.35		

Note: Models include adjustment for age at baseline SCCS interview (continuous) and percentage African ancestry (continuous)

Table A.18. Adjusted means for body mass index (BMI, kg/m2) and p-values from race stratified linear regression models examining BMI and *ADIPOR1* SNPs.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs6672643	A/A	29.97	2	0.028	30.42	2	0.42
	A/G	30.80			29.90		
	G/G	31.74			31.41		
rs2185781	A/A	30.89	2	0.63	29.89	2	0.30
	A/G	30.14			30.83		
	G/G	30.51			30.14		
rs4336908	A/A	30.23	1	0.72	29.89	2	0.39
	A/G				30.78		
	G/G	30.44			30.17		
rs10920531	A/A	30.69	2	0.23	29.78	2	0.15
	A/C	30.55			30.82		
	C/C	29.80			30.08		
rs7539542	C/C	30.74	2	0.10	28.85	2	0.019
	C/G	30.46			30.89		
	G/G	29.47			30.21		
rs1342387	A/A	30.41	2	0.69	30.25	2	0.76
	A/G	30.54			30.23		
	G/G	30.12			30.57		
rs7518457	A/A	30.39					
	A/G	30.62	1	0.71			
rs12045862	A/A	30.29	1	0.77	29.93	2	0.86
	A/G				30.41		
	G/G	30.44			30.35		
rs2275737	A/A	30.09	2	0.34	30.21	2	0.88
	A/C	30.72			30.29		
	C/C	30.15			30.48		
rs12733285	A/A	30.63	2	0.44	30.63	2	0.74
	A/G	30.76			30.16		
	G/G	30.21			30.44		

Table A.18. (continued) Adjusted means for body mass index (BMI, kg/m^2) and p-values from race stratified linear regression models examining BMI and *ADIPOR1* SNPs.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs10753929	A/A	29.44	2	0.59	29.59	2	0.82
	A/G	30.38			30.22		
	G/G	30.49			30.40		
rs1539355	A/A	30.24	2	0.34	30.20	2	0.75
	A/G	30.70			30.43		
	G/G	29.99			30.69		
rs10800888	A/A	30.39	2	0.80			
	A/G	30.66					
	G/G	30.34					
rs6666089	A/A	30.91	2	0.86	30.95	2	0.64
	A/G	30.24			30.30		
	G/G	30.45			30.26		
rs7523903	C/C	30.41	2	0.86			
	C/G	30.28					
	G/G	30.51					
rs2232849	A/A	30.24	1	0.70			
	G/G	30.45					
rs2232844	A/A	30.35	1	0.50			
	A/G	30.71					
rs2232842	A/A	30.50	2	0.10	30.38	1	0.44
	A/G	30.42			29.68		
	G/G	27.58					

Note: Models include adjustment for age at baseline SCCS interview (continuous) and percentage African ancestry (continuous)

Table A.19. Adjusted means for body mass index (BMI, kg/m2) and p-values from race stratified linear regression models examining BMI and SNPs in *ADIPOR2*.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs758027	A/A	30.25	2	0.23			
	A/G	31.15					
	G/G	30.89					
rs1029629	A/A	30.39	2	0.96	30.58	2	0.55
	A/C	30.41			30.11		
	C/C	30.67			30.17		
			1	0.59			
rs7304096	A/A	30.38					
	A/G	30.80					
rs2058033	A/A	30.40	1	0.77	30.48	2	0.51
	A/C	30.67			29.88		
	C/C				30.27		
rs7975600	A/A	33.36	2	0.0085	29.61	2	0.62
	A/T	29.41			30.66		
	T/T	30.68			30.26		
rs11832817	A/A	33.35	2	0.0062	30.23	2	0.91
	A/G	29.53			30.25		
	G/G	30.71			30.43		
rs12826079	A/A				29.89	1	0.42
	G/G				30.41		
rs10773982	A/A	30.52	2	0.22	30.57	2	0.56
	A/G	30.11			30.09		
	G/G	31.16			30.37		
rs11061946	A/A	30.20	1	0.87	30.13	1	0.68
	G/G	30.42			30.38		
rs10773983	A/A	30.57	2	0.55	29.53	2	0.068
	A/G	30.13			29.98		
	G/G	30.72			30.84		
rs12316367	A/A	34.01	2	0.033	30.79	2	0.27
	A/G	30.16			30.31		
	G/G	30.40			29.82		

Table A.19. (continued) Adjusted means for body mass index (BMI, kg/m^2) and p-values from race stratified linear regression models examining BMI and *ADIPOR2* SNPs.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs10773989	A/A	30.25	2	0.28	29.91	2	0.38
	A/G	30.45			30.34		
	G/G	31.60			30.76		
rs2058112	A/A	30.83	2	0.79	27.16	2	0.20
	A/G	30.25			30.12		
	G/G	30.47			30.46		
rs12298275	A/A	30.41	1	0.90			
	A/G	30.50					
rs7134070	A/A	30.41	2	0.96			
	A/G	30.45					
	G/G	30.02					
rs7967137	A/A	30.65	2	0.063	30.46	2	0.28
	A/G	29.91			30.10		
	G/G	31.46			27.75		
rs7138701	A/A	30.47	2	0.92			
	A/G	30.29					
	G/G	30.47					
rs11614639	A/A	30.76	2	0.34	30.65	2	0.24
	A/C	30.40			30.42		
	C/C	29.89			29.65		
rs10773991	A/A	32.26	2	0.21	30.78	2	0.20
	A/G	30.23			30.38		
	G/G	30.41			29.71		
rs4140993	A/A	30.51	2	0.55			
	A/C	30.09					
	C/C	31.11					
rs16928751	A/A	31.02	2	0.53	27.75	2	0.28
	A/G	30.13			30.10		
	G/G	30.53			30.46		

Table A.19. (continued) Adjusted means for body mass index (BMI, kg/m^2) and p-values from race stratified linear regression models examining BMI and SNPs in *ADIPOR2*.

		Black women			White women		
SNP	**Genotype**	**BMI mean**	**df**	**p-value**	**BMI mean**	**df**	**p-value**
rs2286384	C/C	31.28	2	0.11	30.79	2	0.20
	C/G	30.69			30.37		
	G/G	29.98			29.71		
rs12342	A/A	29.05	2	0.040	30.33	2	0.57
	A/G	29.79			30.08		
	G/G	30.78			30.56		
rs1044471	A/A	33.71	2	0.0040	30.58	2	0.48
	A/G	30.53			30.45		
	G/G	30.16			29.92		
rs7294540	A/A	30.41	2	0.23	29.51	2	0.12
	A/C	30.25			30.33		
	C/C	33.16			30.80		
rs13219	A/A	32.96	2	0.15	30.74	2	0.15
	A/G	30.20			30.37		
	G/G	30.42			29.56		
rs2058111	A/A	30.37	2	0.34	29.58	2	0.17
	A/C	30.33			30.36		
	C/C	32.10			30.74		

Note: Models include adjustment for age at baseline SCCS interview (continuous) and percentage African ancestry (continuous).

Table A.20. Final multiple linear regression model results for adiponectin-BMI model after confounder adjustment for entire study sample (N=1,992) and subset of women who gave a fasting blood sample (N=870).

	Entire sample			Fasting subset		
	Beta	**Std err**	**p-value**	**Beta**	**Std err**	**p-value**
WHITE WOMEN						
Body Mass Index (kg/m^2)	-0.030	0.003	<.0001	-0.030	0.005	<.0001
Age at interview (years)	0.014	0.002	<.0001	0.018	0.004	<.0001
HDL-cholesterol (mg/dl)						
Q1 (<43)	Referent					
Q2 (43-50)	0.30	0.053	<.0001	0.22	0.083	0.009
Q3 (51-60)	0.42	0.057	<.0001	0.39	0.086	<.0001
Q4 (>60)	0.68	0.062	<.0001	0.75	0.10	<.0001
BLACK WOMEN						
Body Mass Index (kg/m^2)	-0.018	0.004	<.0001	-0.012	0.005	0.02
Age at interview (years)	0.009	0.002	0.0003	0.006	0.004	0.13
HDL-cholesterol (mg/dl)						
Q1 (<43)	Referent					
Q2 (43-50)	0.17	0.067	0.01	0.20	0.10	0.045
Q3 (51-60)	0.53	0.067	<.0001	0.61	0.10	<.0001
Q4 (>60)	0.59	0.064	<.0001	0.59	0.10	<.0001

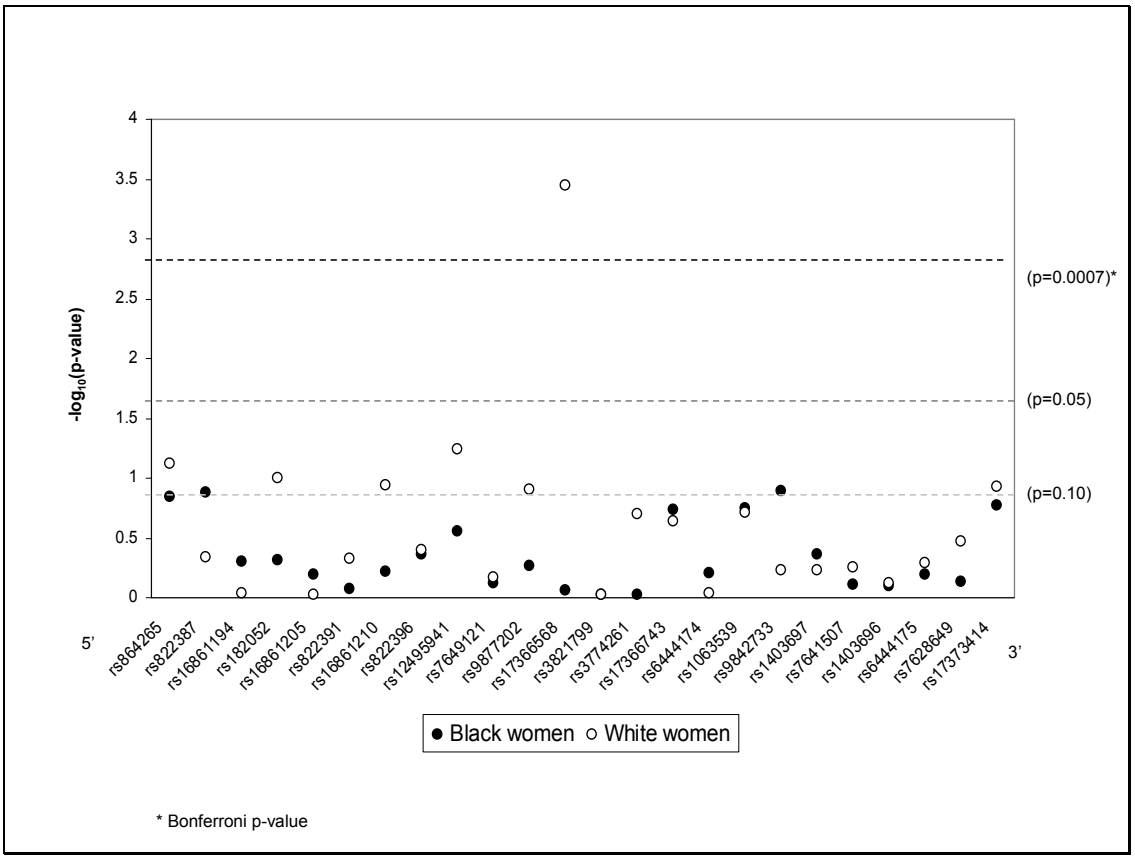

Figure A.1a. - log10(p-value) from linear regression models with log-adiponectin as outcome and SNPs in *ADIPOQ* as exposure of interest among black and white women. Models were adjusted for age at interview and percentage of African ancestry.

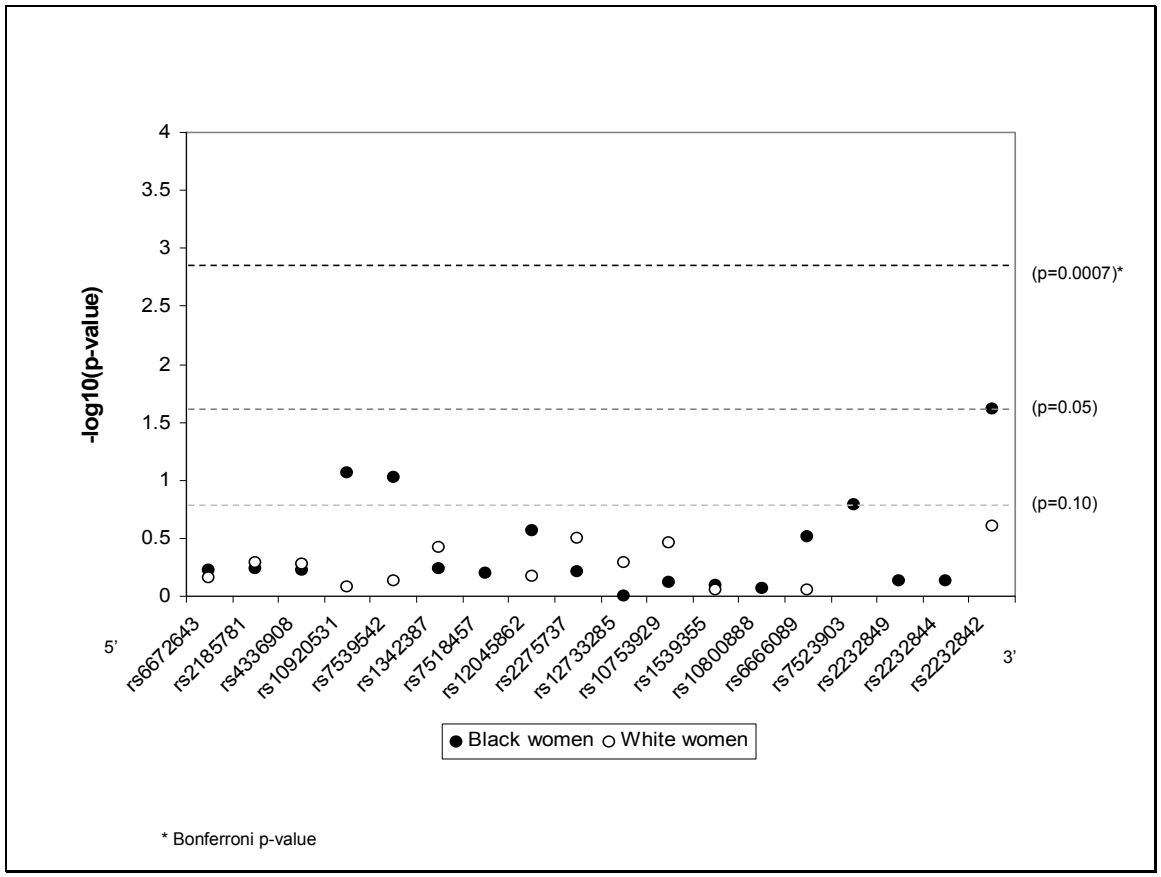

Figure A.1b. - log10(p-value) from linear regression models with log-adiponectin as outcome and SNPs in *ADIPOR1* as exposure of interest among black and white women. Models were adjusted for age at interview and percentage of African ancestry.

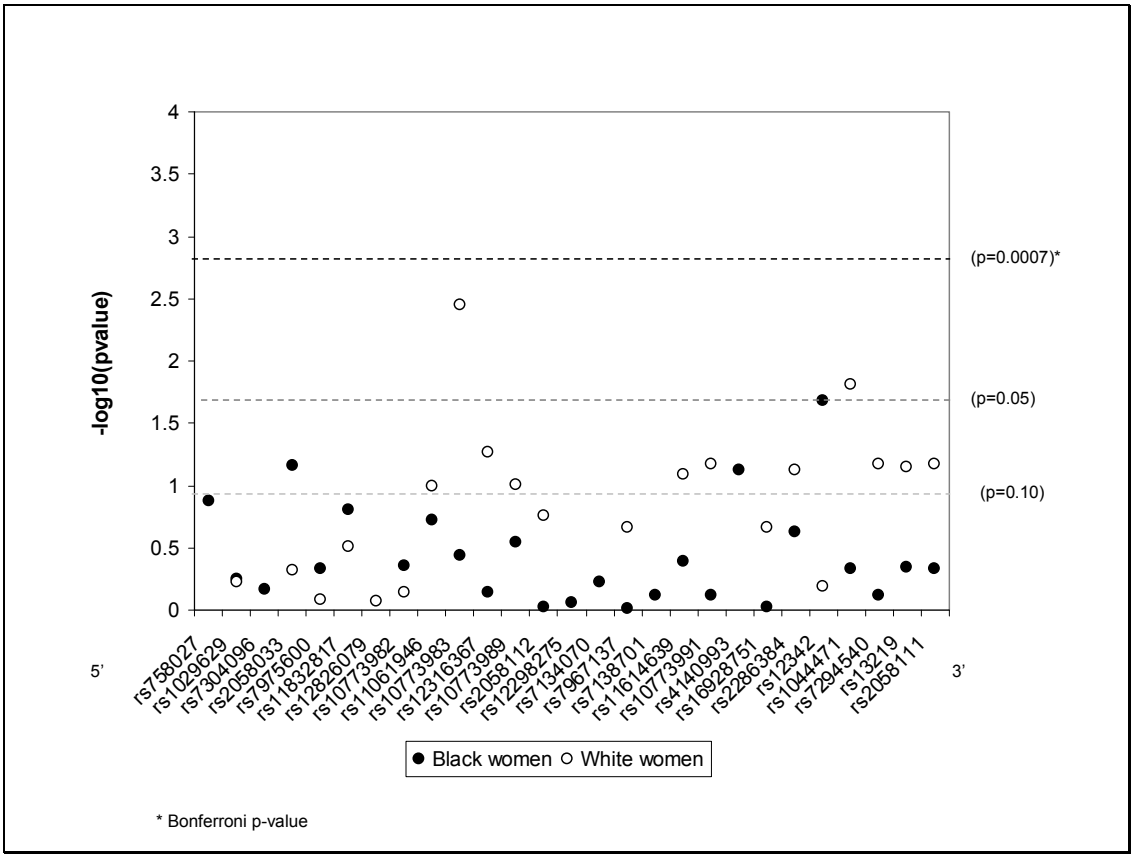

Figure A.1c. - log10(p-value) from linear regression models with log-adiponectin as outcome and SNPs in *ADIPOR2* as exposure of interest among black and white women. Models were adjusted for age at interview and percentage of African ancestry.

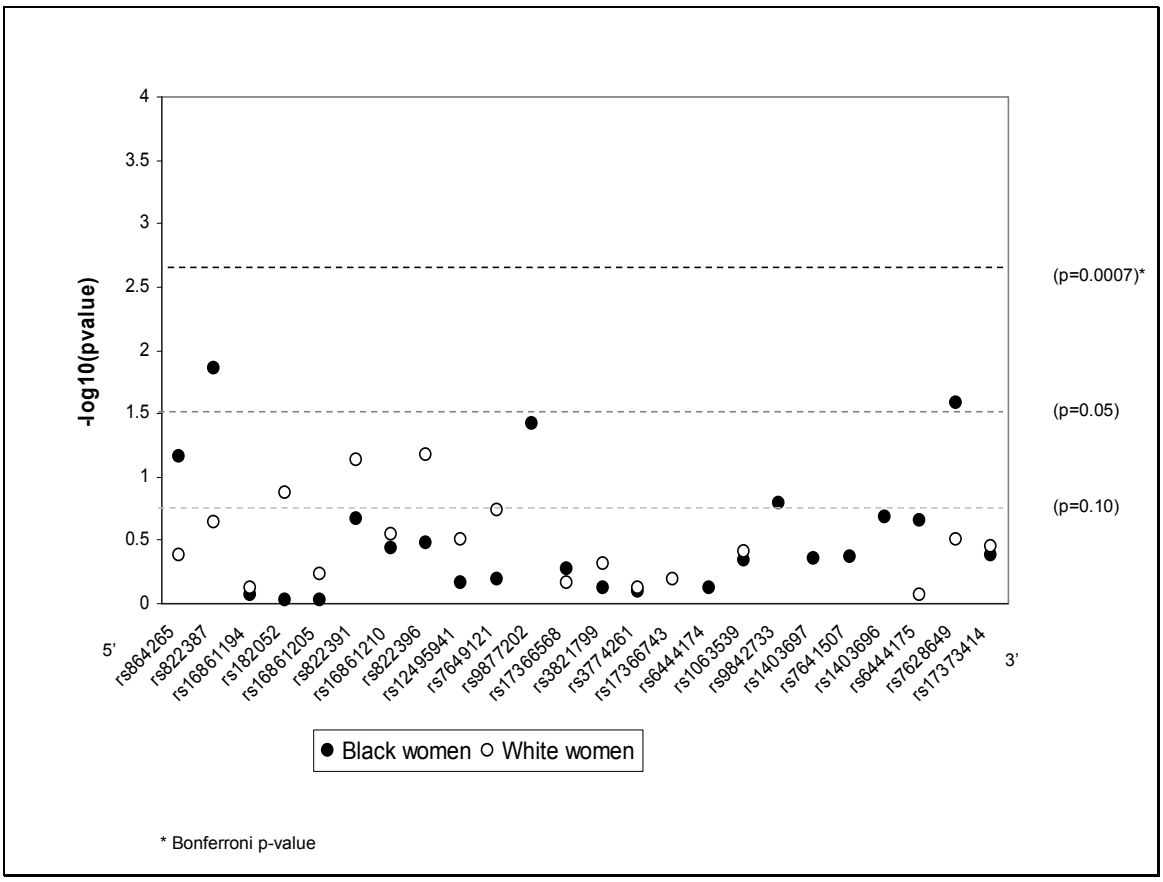

Figure A.2a. - log10(p-value) from linear regression models with body mass index (BMI) as outcome and SNPs in *ADIPOQ* as exposure of interest among black and white women. Models were adjusted for age at interview and percentage of African ancestry.

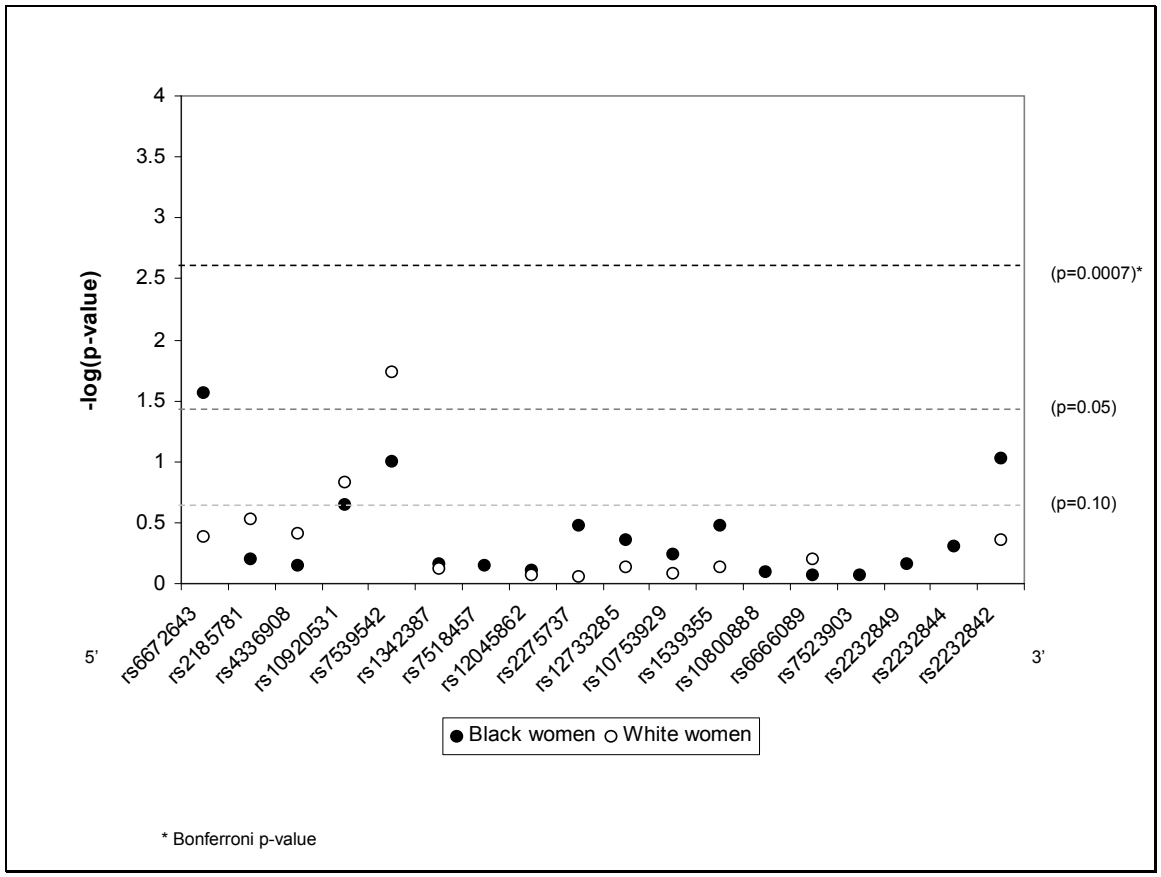

Figure A.2b. - log10(p-value) from linear regression models with body mass index (BMI) as outcome and SNPs in *ADIPOR1* as exposure of interest among black and white women. Models were adjusted for age at interview and percentage of African ancestry.

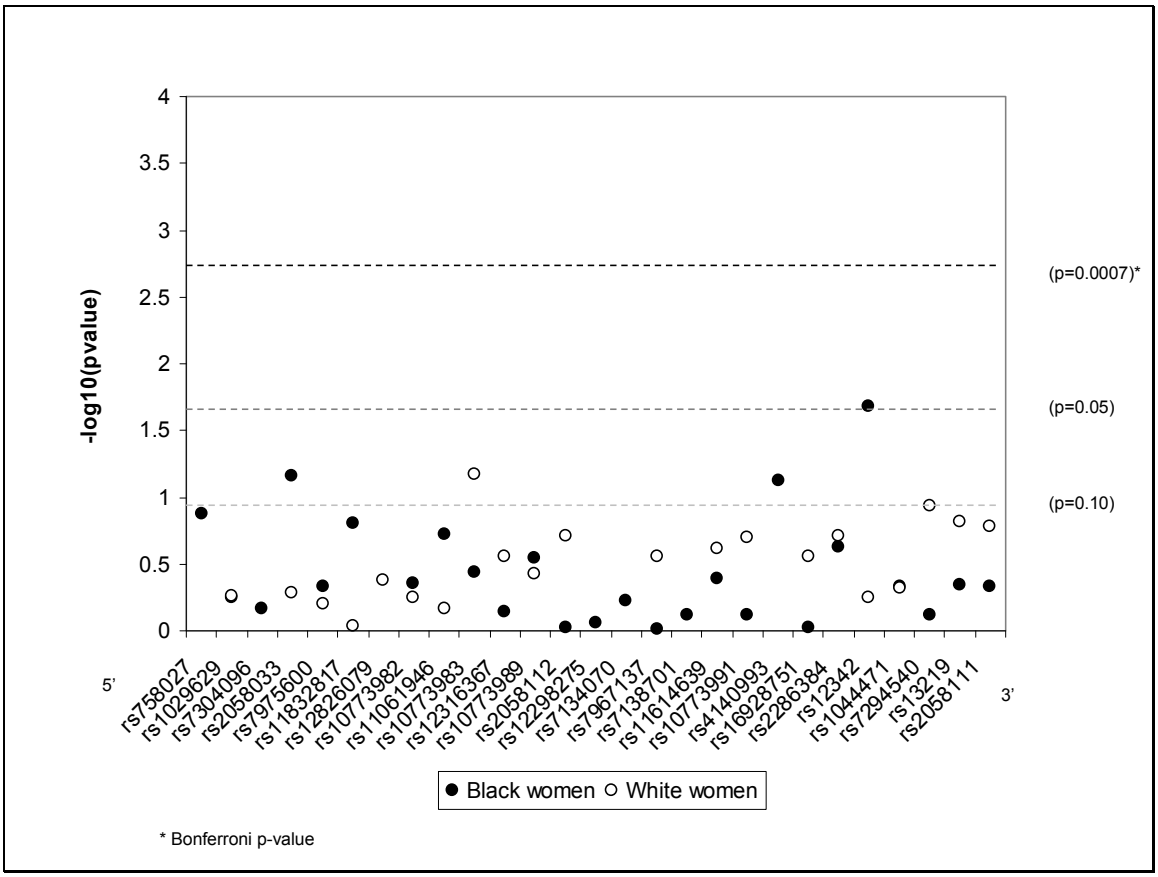

Figure A.2c. - log10(p-value) from linear regression models with body mass index (BMI) as outcome and SNPs in *ADIPOR2* as exposure of interest among black and white women. Models were adjusted for age at interview and percentage of African ancestry